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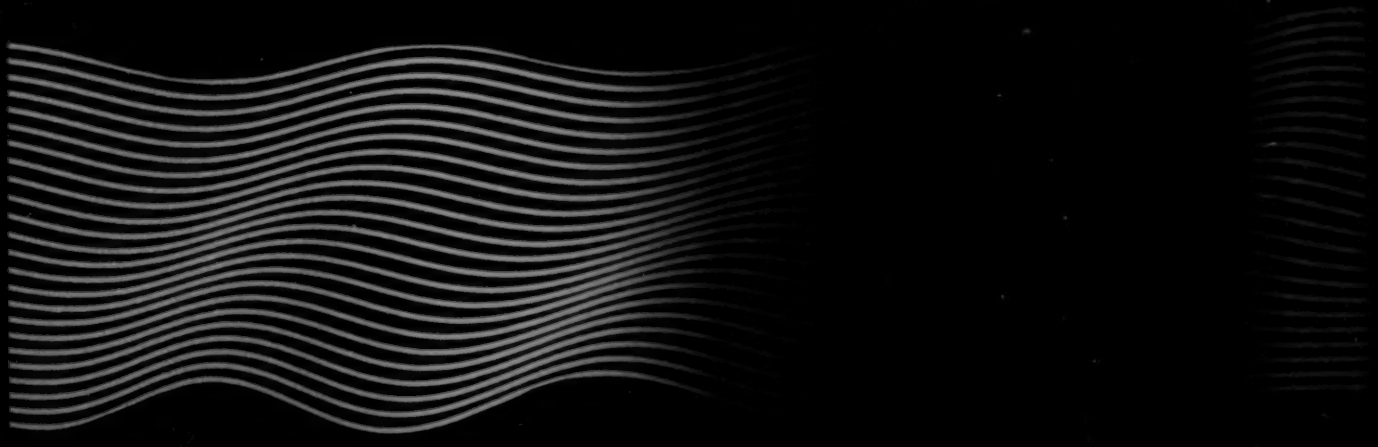

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Influences of temperature upon the postexercise physiology of Atlantic salmon (*Salmo salar*)

Michael P. Wilkie, Mark A. Brobbel, Kevin Davidson, Leonard Forsyth, and Bruce L. Tufts

Abstract: Atlantic salmon (*Salmo salar*) were acclimated and exhaustively exercised at 12, 18, or 23°C to determine how temperature influences the magnitude of postexercise physiological disturbances. At each temperature, exercise led to decreased white muscle ATP and phosphocreatine concentrations. Phosphocreatine was rapidly restored within 1 h at each temperature whereas ATP restoration took 1–4 h at 18 and 23°C, but considerably longer at 12°C. Exercise-induced depletions of white muscle glycogen were accompanied by elevations in muscle lactate, which contributed to 0.6 unit decreases in white muscle intracellular pH (pH_i) at each temperature. Compared with rates of recovery in warmer water, glycogen resynthesis, lactate catabolism, and pH_i correction were slower at 12°C. White muscle REDOX state estimates suggested that slower postexercise recovery at 12°C was not due to oxygen delivery limitations. Marked postexercise elevations in plasma osmolality and lactate concentration were also observed and in each case correction of the disturbance took longer at 12°C. Paradoxically, significant mortality (30%) was observed only at 23°C. We conclude that while warmer water facilitates postexercise recovery of white muscle metabolic and acid–base status in Atlantic salmon, extremely high temperatures may make them more vulnerable to delayed postexercise mortality.

Résumé : Des saumons atlantiques (*Salmo salar*) ont été acclimatés et soumis à un exercice intensif à 12, 18 ou 23°C; l'étude avait pour but de déterminer comment la température influe sur l'ampleur des perturbations physiologiques post-exercice. Pour chaque température, l'exercice a provoqué une baisse des concentrations d'ATP et de phosphocréatine dans le muscle blanc. La concentration de phosphocréatine a rapidement remonté, en moins de 1 h, à chaque température, tandis que le rétablissement de l'ATP a pris de 1 à 4 h à 18 et à 23°C, mais beaucoup plus longtemps à 12°C. Les déplétions de glycogène du muscle blanc induites par l'exercice s'accompagnaient de hausses du lactate musculaire, qui se traduisaient par des baisses de 0,6 unité du pH intracellulaire (pH_i) du muscle blanc à chaque température. Par rapport aux taux de récupération en eau plus chaude, la resynthèse du glycogène, le catabolisme du lactate et la correction du pH_i étaient plus lents à 12°C. Les estimations de l'état d'oxydoréduction du muscle blanc permettent de penser que la lenteur de la récupération à 12 °C n'était pas due à des limitations de l'apport en oxygène. Des hausses post-exercice marquées de l'osmolalité et de la teneur en lactate du plasma ont aussi été observées et, dans chaque cas, la correction de la perturbation a été plus longue à 12°C. Paradoxalement, une mortalité importante (30%) a été observée à 23°C seulement. Nous en concluons que, si l'eau chaude facilite le rétablissement post-exercice du métabolisme du muscle blanc et du rapport acide-base chez le saumon atlantique, des températures extrêmement élevées peuvent rendre ce poisson plus vulnérable à une mortalité post-exercice à retardement. [Traduit par la Rédaction]

Introduction

As a result of the growing popularity of angling, many populations of North American gamefish are under ever increasing pressure from recreational anglers. To reduce angling mortality and conserve gamefish populations, catch and release or nonconsumptive angling has been widely promoted in recent years. Indeed, stock declines of Atlantic salmon (*Salmo salar*) have prompted the Canadian government to enact legislation that makes catch and release of large adults (>63 cm fork length) mandatory in Canada's Atlantic provinces. However, relatively little is known about the potential impacts that catch and release angling has on these highly prized game fish after their release.

The physiological disturbances that fish experience following angling are considerable (Wydoski et al. 1976; Beggs et al. 1980; Gustaveson et al. 1991; Pankhurst and Dedual 1994; Booth et al. 1995; Kieffer et al. 1995) and are often characterized by complete physical exhaustion (Kieffer et al. 1995). It is also well established that exhaustive exercise in the laboratory results in marked disturbances to acid–base, osmotic, and electrolyte balance and reductions in high-energy phosphogen fuels, such as ATP and phosphocreatine (e.g., Milligan and Wood 1986*a*, 1986*b*; Dobson et al. 1987; Pearson et al. 1990; Wang et al. 1994*a*, 1994*b*). In addition, exhaustive exercise is usually characterized by an almost complete depletion

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of muscle glycogen stores and large increases in muscle and blood lactate concentration (Black 1957; Black et al. 1962; Milligan and Wood 1986*b*; Dobson et al. 1987; Pearson et 1990; Wang et al. 1994*a*). In some cases, postexercise physiological disturbances may be severe enough to result in delayed postangling–exercise mortality (Black 1958; Bouck and Ball 1966; Beggs et al. 1980; Wood et al. 1983; Ferguson and Tufts 1992).

In contrast to Atlantic salmon angled in the late fall at 6°C, we recently found that returning Atlantic salmon angled under midsummer (~20°C) conditions were unable to resynthesize glycogen or fully restore white muscle intracellular pH to resting levels within the experimental recovery period (4 h; Wilkie et al. 1996). Moreover, all of the salmon angled in the late fall survived (Booth et al. 1995), but survival under midsummer conditions was only 60% (Wilkie et al. 1996). Although several factors, such as the duration of freshwater acclimation and degree of starvation, also differed between the salmon angled in the summer and fall, we suggested that environmental temperature may have played an important role in the different postangling physiological responses that were observed. Indeed, angling at elevated water temperatures (e.g., >20°C) is known to exacerbate ionoregulatory and osmotic disturbances in the blood of rainbow trout (*Oncorhynchus mykiss*; Wydoski et al. 1976) and largemouth bass (*Micropterus salmoides*; Gustaveson et al. 1991). Accordingly, the objective of the present investigation was to examine the influence of temperature on the postexercise physiology and survival of mature Atlantic salmon that were fully acclimated to fresh water, had identical life histories, and, until just prior (2 weeks) to the experiments, were held at similar water temperatures.

Materials and methods

Experimental animals and setup

Experiments were conducted at the Margaree Fish Culture Station (MFCS), Margaree Valley, Nova Scotia, in August 1994 on sexually mature hatchery-reared Atlantic salmon (mass 2.03 ± 0.05 kg (mean ± SEM); length 60.7 ± 0.5 cm). Approximately 2 weeks before the experiments, the fish were transferred from outdoor, freshwater holding ponds (pH 7.1; $[Ca^{2+}]$, 1.4 mequiv.·L^{-1}; $[Mg^{2+}]$, 0.3 mequiv.·L^{-1}; $[Na^{+}]$, 3.7 mequiv.·L^{-1}; $[Cl^{-}]$, 3.8 mequiv.·L^{-1}; alkalinity, 14.5 mequiv.·L^{-1}) to the MFCS's indoor facilities, where the salmon were equally distributed ($n = 26$), to three large fibreglass Swede (holding) tanks (2.4 × 2.4 × 1.0 m) with a water depth of 0.8 m. There was a continual inflow (about 10 L·min^{-1}) of brook water, identical in chemical composition to that in the holding ponds, and temperature was initially maintained at 18°C. The fish were allowed to adjust to their new surroundings for 3–5 days, after which water temperatures were adjusted toward the nominal experimental temperatures of 12 or 23°C in two of the three tanks at a rate of 1°C·day^{-1}; the remaining tank was regulated at 18°C. The 23°C water was maintained by mixing heated brook water with normal, cooler brook water. The 12°C water was pumped from a well and had a similar composition to the brook water (pH, 7.5; $[Ca^{2+}]$, 2.8 mequiv.·L^{-1}; $[Mg^{2+}]$, 1.1 mequiv.·L^{-1}; $[Na^{+}]$, 2.6 mequiv.·L^{-1}; alkalinity, 1.5 mequiv.·L^{-1}).

Feeding was terminated after the fish were transported indoors because appetite might have been differentially influenced by the different temperature regimes and because migrating Atlantic salmon naturally stop feeding upon entering fresh water (Scott and Crossman 1973; Tufts et al. 1991). After the experimental temperatures were achieved, the fish were left undisturbed in their tanks for a minimum of 2 weeks before postexercise physiology was assessed, and the total period of starvation approximated 3–5 weeks.

The system designed to assess the postexercise physiology of the salmon consisted of two large wooden boxes that contained four individual chambers per box. One day prior to experimentation the salmon were netted, one at a time, from the appropriate holding tanks (12, 18, or 23°C), placed in an individual chamber, and allowed to recover overnight at the appropriate temperature. The covered chambers were large enough to permit the salmon to move about (dimensions 22 cm deep × 27 cm wide × 120 cm long) and contained about 70 L of water. Water was directly pumped to the boxes from the appropriate holding tanks and flow rates into each chamber approximated 2.75 ± 0.25 L·min^{-1}. Oxygenation was maintained at 90–100% saturation with air stones connected to a Summit 5 artificial oxygenator (Mountain Medical Equipment Inc., Littleton, Colo.).

Experimental protocols

This study was divided into two distinct series. In part 1, salmon were exercised to exhaustion and then terminally sampled for analysis of physiological variables, at 12, 18, or 23°C. Resting fish, which were sampled in an identical manner to the exercised animals, served as the controls. In part 2, survival was monitored for 3 days following exhaustive exercise at one of the three temperatures. In both series of experiments, exercised fish were chased manually, one at a time, for 6 min in a 1.5 m diameter circular exercise tank containing 30–40 cm of water at the appropriate temperature.

Part 1: postexercise physiology

After being allowed to acclimate overnight to the holding boxes, the fish were transferred, one at a time, to the exercise tank where they were exhaustively exercised for 6 min. The fish were then anaesthetized in MS-222 (see below) and immediately sampled (0 h), or returned to their respective holding chambers and allowed to recover for 1 or 4 h. Resting fish, which were also held in the holding boxes and subsequently anaesthetized, served as the controls. Anaesthetization was achieved by directly adding concentrated MS-222 (1 g·L^{-1}) solution, buffered with $NaHCO_3^-$ (2 g·L^{-1}) , to the water in each holding chamber. This yielded a final concentration of 0.1 g·L^{-1} MS-222 in each chamber. When the fish were unresponsive (within 1 min) they were quickly transferred to a box containing concentrated MS-222 (0.3 g·L^{-1}) for 1 min to ensure complete anaesthetization, after which blood and tissue samples were taken within 30 s. Confinement in fish boxes and subsequent anaesthetization with buffered MS-222 was chosen because it is most effective for preserving muscle and blood metabolite concentrations, such as lactate, ATP, phosphocreatine (PCr), and glycogen, in fishes (Tang and Boutilier 1991; Wang et al. 1994*b*).

After anaesthetization, 10-mL blood samples were withdrawn into heparinized, plastic disposable syringes via caudal puncture and immediately placed on ice until muscle sampling was complete. White muscle was then excised from the dorsal region of the trunk, just anterior to the dorsal fin, and quick frozen with aluminum tongs cooled with liquid N_2 and immediately plunged into liquid N_2. Blood samples were then centrifuged at 10 000 × *g* for 3 min, and the resultant supernatant was withdrawn and immediately frozen for later determination of plasma lactate and osmolality. Muscle samples were stored in liquid N_2 for future analysis of muscle pH_i, water content, glycogen, ATP, PCr, free creatine, lactate, and pyruvate. Immediately following blood and muscle sampling, the fish were killed by a sharp blow to the head.

Part 2: postexercise survival

This experiment was designed to determine if exercise resulted in any delayed mortality at each of the experimental temperatures. At each temperature, fish ($n = 10$; experimental) were netted one at a time from their respective holding tanks and were then transferred to the exercise tank and chased for 6 min. Efforts were always made to

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minimize air exposure (<10 s) because it is known to exacerbate exercise-induced physiological disturbances and could contribute to delayed postexercise mortality (Ferguson and Tufts 1992). Immediately following exercise, the fish were transferred to separate, but otherwise identical, holding tanks that were maintained at the appropriate temperatures and survival was monitored for 3 days. We avoided returning the fish to their tank of origin because any mortality or exercise-induced stress might have unduly stressed the resting fish, which served as the controls ($n = 16$).

Analytical techniques

White muscle pH_i was determined according to methods outlined in Pörtner et al. (1990). Briefly, frozen chunks of muscle tissue were ground to a fine powder under liquid N_2 and approximately 200 mg of the powder was added to 800 µL of metabolic inhibitor containing 150 mmol·L^{-1} of KF and 6 mmol·L^{-1} of the disodium salt of nitrilotriacetic acid. The mixture was then vortexed and briefly centrifuged, and the pH of the supernatant was subsequently measured on a thermostatted Radiometer capillary pH electrode, which was connected to a Radiometer PHM84 pH meter.

Sample processing for metabolite analyses entailed grinding 1.0-g chunks of muscle to a fine powder under liquid N_2 followed by protein extraction in four volumes of 8% perchloric acid, containing 1 mmol·L^{-1} ethylenediaminetetraacetic acid. Care was taken to ensure that the powdered samples were not contaminated with connective tissue or blood. The acidified extract was continuously mixed for 5 min and centrifuged at 10 000 × g for 2 min at 4°C, and the supernatant was drawn off and neutralized with 1.5 volumes of 2 mol·L^{-1} KOH, containing 0.4 mol·L^{-1} imidazole and 0.4 mol·L^{-1} KCl. After this latter solution was quickly mixed, it was centrifuged for 30 s, and the resultant supernatant was dispensed into 1.5-mL centrifuge tubes and stored frozen in liquid N_2. These extracts were later analyzed enzymatically for ATP, free creatine, phosphocreatine, lactate, and pyruvate according to Bergmeyer (1983). Muscle glycogen was determined enzymatically, using the amyloglucosidase–hexokinase assay described in Hassid and Abraham (1957), on neutralized extracts of muscle that were digested in five volumes of 30% KOH. Muscle H_2O content was determined by oven drying 500 mg of powdered white muscle to constant mass at 80°C.

Plasma lactate was determined enzymatically using lactate dehydrogenase (Bergmeyer 1983). Plasma osmolality was determined with a clinical osmometer (model 3MO Plus, Advanced Instruments Inc.). Blood haemoglobin concentration was determined using a commercial kit (cyanmethaemoglobin method; Sigma Chemical Co., St. Louis, Mo.).

Calculations and statistics

Muscle data were expressed in millimoles per litre of tissue water after dividing the wet tissue mass (millimoles per kilogram of wet tissue) by the muscle water content (litres of tissue water per kilogram of wet tissue). Estimates of the mean erythrocyte haemoglobin concentration (MCHC) were determined by dividing the whole blood haemoglobin concentration by the haematocrit. The REDOX state of the muscle (REDOX = NAD^+/NADH ratio) was estimated according to Wang et al. (1994*a*) and is described by the following formula:

$$(1) \quad [NAD^+]/[NADH] = ([pyruvate][H^+])/([lactate]\ K_{LDH})$$

where [pyruvate] is the white muscle pyruvate concentration (millimoles per litre of tissue water), $[H^+]$ is the concentration of intracellular protons, [lactate] is the white muscle lactate concentration, and K_{LDH} is the equilibrium constant for the lactate dehydrogenase reaction as provided by Williamson et al. (1967) and corrected for temperature using the van't Hoff equation (Williams and Williams 1967).

Data generated in part 1 were expressed as the mean ± SEM. When significant variation was indicated by analysis of variance (ANOVA), data were analyzed using a Tukey–Kramer post-test at the $P < 0.05$ level. In instances where data did not follow a normal distribution, a nonparametric ANOVA test was used followed by a Dunnet's post-test. Data from the postexercise survival experiment were analyzed using contingency tables and significance ($P < 0.05$) was determined using Fisher's exact test.

Results

Part 1: postexercise physiology

Muscle metabolites and acid–base status

White muscle ATP concentrations were about 15 mmol·L^{-1} in all three groups of resting salmon (Fig. 1A). Immediately following exercise, ATP had decreased by 70% at 12°C, by 40% at 18°C, and by 50% at 23°C. ATP concentrations quickly returned to resting levels in the fish sampled at 23°C, but at 12 and 18°C the rates of recovery were slower. By 4 h, ATP approached resting concentrations at 18°C, but ATP was still 45% below resting values at 12°C (Fig. 1A).

Resting PCr concentrations averaged 50 mmol·L^{-1} at 12 and 23°C but were slightly lower at 18°C (Fig. 1B). Exercise resulted in a significant depletion of PCr at all three temperatures, but reductions were most pronounced at 12°C, where PCr decreased 85%. Lesser (50%) reductions were observed at 18 and 23°C (Fig. 1B). In all cases, PCr reductions were matched by proportionate increases in the free creatine concentration (data not shown). These marked changes in PCr concentration were not, however, accompanied by significant changes in the total creatine pool in salmon white muscle. The total creatine pool, estimated from measurements of phosphocreatine and free creatine, averaged 70 mmol·L^{-1} in resting fish sampled at all three experimental temperatures (data not shown). Approximately 65% of the total creatine pool was phosphorylated at 12 and 23°C, and 56% of the pool was phosphorylated at 18°C (data not shown).

Resting white muscle glycogen concentrations were about 25 mmol·L^{-1} in fish held at 18 and 23°C but were significantly lower, by approximately 40%, in fish maintained at 12°C (Fig. 1C). Regardless, exercise resulted in a near depletion of glycogen reserves in all three groups of fish (Fig. 1C). Temperature had a profound effect on the rate of glycogen replenishment. After 4 h, fish exercised at 12°C had only replenished about 30% of their resting reserves. Fish at 18°C showed a more rapid recovery and had resynthesized about 45% of their missing glycogen by 4 h, whereas fish at 23°C had recovered more than 80% of their resting reserves by this time (Fig. 1C).

White muscle lactate concentrations were between 3 and 5 mmol·L^{-1} in resting fish at all three temperatures (Fig. 2A) and exercise resulted in peak lactate concentrations that exceeded 50 mmol·L^{-1} in each group. By 1 h, about 50% of the white muscle lactate load had been metabolized by fish held at 18 and 23°C, but no change was observed in the lactate load of the 12°C salmon (Fig. 2A). By 4 h the lactate load was approaching resting concentrations in the 18 and 23°C salmon but was still significantly elevated, by sixfold, in the 12°C group (Fig. 2A).

The intracellular lactacidosis was associated with a significant increase in plasma lactate concentration. Resting plasma lactate concentrations were less than 1 mmol·L^{-1} at all three temperatures, but immediately following exercise, concentrations increased markedly and approached 9 mmol·L^{-1} in all three groups of fish (Fig. 2B). After 1 h, plasma lactate concentrations had increased by an additional 30% in those fish

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Fig. 1. Changes in the white muscle (A) ATP ([ATP]), (B) phosphocreatine ([PCr]), and (C) glycogen ([glycogen]) concentrations of Atlantic salmon following exhaustive exercise at 12 (open bars), 18 (hatched bars), or 23°C (solid bars). The vertical stippled bar represents the period of exercise (EX). All data are expressed as the mean ± 1 SEM; $n = 5–8$ at rest and following exercise at each temperature. However, $n = 2$ after 4 h at 23°C because of delayed postexercise mortality in four of the six fish exercised. *, significant differences from resting values at each respective temperature; †, significant differences from values for 12°C fish; ‡, significant differences from values for 18°C fish ($P < 0.05$).

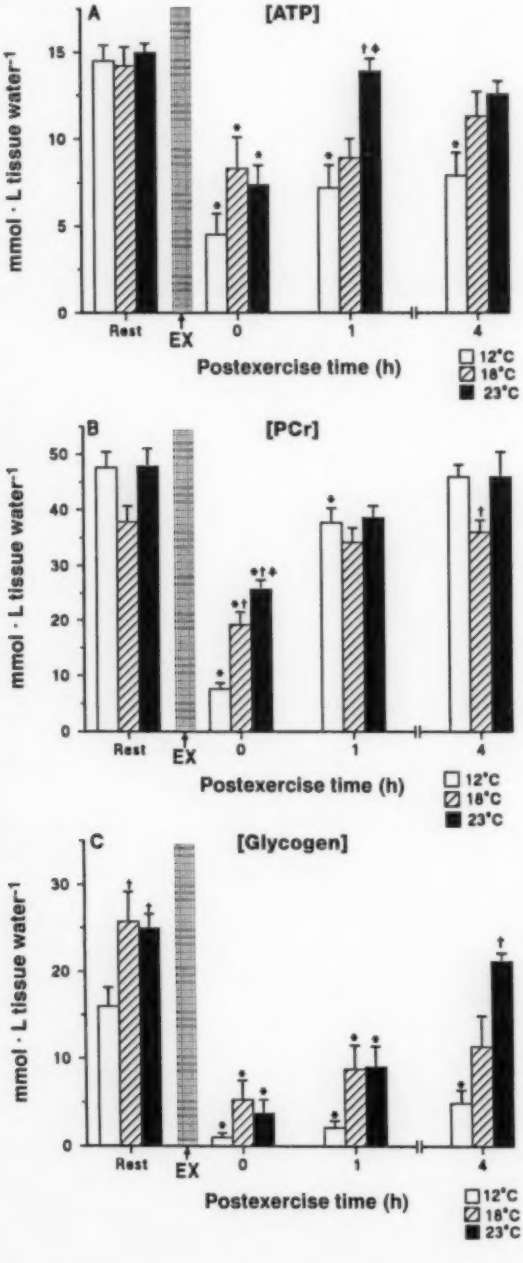
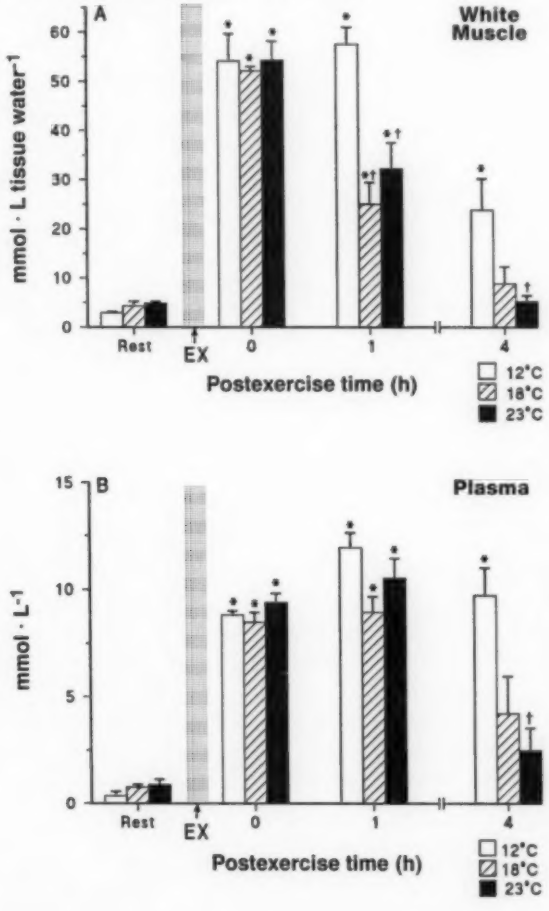


Fig. 2. Changes in the (A) white muscle lactate and (B) plasma lactate concentrations of Atlantic salmon following exhaustive exercise at 12 (open bars), 18 (hatched bars), or 23°C (solid bars). The vertical stippled bar represents the period of exercise (EX). All data are expressed as the mean ± 1 SEM; $n = 5–8$ at rest and following exercise at each temperature. However, $n = 2$ after 4 h at 23°C owing to delayed postexercise mortality in four of the six fish exercised. *, significant differences from resting values at each respective temperature; †, significant differences from values for 12°C fish in each sampling period ($P < 0.05$).



recovering at 12°C. By 4 h, most of the plasma lactate load had been cleared in the 18 and 23°C salmon, but virtually none of it had been cleared from the plasma of salmon exercised at 12°C (Fig. 2B).

Muscle pyruvate concentrations in resting fish approximated 0.08 mmol·L^{-1} at 12 and 18°C but were slightly higher in fish held at 23°C (Table 1). Exercise resulted in an immediate, eightfold increase in muscle pyruvate concentrations at 12°C and slightly less pronounced elevations at 18 and 23°C. By 1 h, pyruvate concentrations were approaching resting levels in the 18 and 23°C salmon but increased by an additional 50%, to approximately 0.9 mmol·L^{-1}, in the fish recovering at 12°C. By 4 h, muscle pyruvate had returned to resting concentrations in all groups (Table 1).

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Table 1. Changes in pyruvate concentration, the [pyruvate]/[lactate] ratio, and the estimated REDOX state (REDOX: $[NAD^+]/[NADH]$ ratio) in the white muscle of exhaustively exercised Atlantic salmon at 12, 18, and 23°C.

		[Pyruvate] ($mmol \cdot L^{-1}$)	[Pyruvate]/[lactate]	$[NAD^+]$/[NADH]
12°C	Rest	0.07±0.02	0.029±0.008	797±208
	0 h postexercise	0.59±0.05*	0.011±0.001*	1506±202
	1 h postexercise	0.90±0.04*	0.016±0.000	2535±195
	4 h postexercise	0.25±0.09	0.011±0.003*	917±242
18°C	Rest	0.09±0.02	0.022±0.004	499±70
	0 h postexercise	0.51±0.16*	0.009±0.003*	793±302
	1 h postexercise	0.38±0.06†	0.016±0.002	752±93†
	4 h postexercise	0.14±0.02	0.033±0.010	788±232
23°C	Rest	0.19±0.02†‡	0.039±0.004	613±62
	0 h postexercise	0.44±0.07	0.008±0.001*	488±73†
	1 h postexercise	0.40±0.11†	0.012±0.002*	536±149†
	4 h postexercise	0.20±0.04	0.029±0.010	609±6

Note: Values are given as the mean ± 1 SE (n = 5–8, except after 4 h at 23°C where n = 2).

*Significant difference from resting values ($P < 0.05$) at each respective temperature.

†Significant difference form 12°C fish ($P < 0.05$) for each sampling period.

‡Significant difference from 18°C fish ($P < 0.05$) for each sampling period.

Exercise resulted in a significant acidosis in the white muscle intracellular compartment (ICF). Resting white muscle pH_i was approximately 7.30 at 12°C and about 7.20 at 18 and 23°C (Fig. 3). Exercise resulted in similar 0.5- to 0.6-unit depressions in pH_i at all three temperatures, but the patterns of pH_i correction were markedly different thereafter. At 12°C, pH_i decreased further, by about 0.1 units, during the first 1 h postexercise. At the upper two temperatures, muscle pH_i gradually recovered, and by 4 h it had returned to resting levels. In contrast, the fish recovering at 12°C had pH_i values that were still 0.4 units below resting values after 4 h of postexercise recovery (Fig. 3).

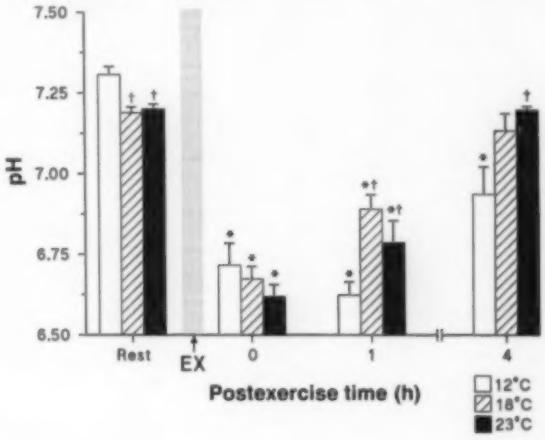
Estimates of white muscle REDOX, calculated from the muscle lactate, pyruvate, and H^+ concentrations, are presented in Table 1. The resting REDOX values were similar at all three temperatures and ranged between 500 and 800 (Table 1). There was a threefold increase in postexercise REDOX after 1 h at 12°C, but REDOX had returned to base-line levels after 4 h. REDOX was unchanged following exercise at 18 and 23°C (Table 1).

In contrast to the REDOX estimates, the [pyruvate]/[lactate] ratios were markedly reduced following exercise. The resting [pyruvate]/[lactate] ratio averaged 0.029 at 12°C, 0.022 at 18°C, and 0.039 at 23°C. Following exercise the [pyruvate]/[lactate] ratio was reduced by 60–80% at all three temperatures, with the largest changes occurring at 23°C (Table 1). By 4 h, the [pyruvate]/[lactate] ratio had returned to resting levels in fish recovering at the two upper temperatures, but it was unchanged from 0-h values in the 12°C fish (Table 1).

Osmoregulation

Plasma osmolality in resting salmon averaged about 325 $mosmol \cdot kg^{-1}$ in fish held at 12 and 18°C but it was about 20 $mosmol \cdot kg^{-1}$ higher in fish sampled at 23°C (Table 2). Exercise resulted in markedly elevated osmolality at all three temperatures.

Fig. 3. Changes in the white muscle intracellular pH (pH_i) of Atlantic salmon following exhaustive exercise at 12 (open bars), 18 (hatched bars), or 23°C (solid bars). The vertical stippled bar represents the period of exercise (EX). All data are expressed as the mean ± 1 SEM; n = 5–8 at rest and following exercise at each temperature. However, n = 2 after 4 h at 23°C owing to delayed postexercise mortality in four of the six fish exercised. *, significant differences from resting values at each respective temperature; †, significant differences from 12°C fish in each sampling period ($P < 0.05$).



Immediately following exercise, plasma osmolality increased by about 30 $mosmol \cdot kg^{-1}$ at 12°C, by 46 $mosmol \cdot kg^{-1}$ at 18°C, and by 33 $mosmol \cdot kg^{-1}$ at 23°C. At the upper two temperatures these plasma osmotic disturbances appeared to have been fully corrected by 1 h, but at 12°C the plasma osmolality increased by an additional 30 $mosmol \cdot kg^{-1}$. The osmotic disturbance appeared to be corrected by 4 h in this latter group (Table 2).

The postexercise increases in plasma osmolality were accompanied by significant haemoconcentration in the extracellular fluid compartment of the salmon at all three temperatures. MCHC indicated that there was considerable postexercise red blood cell swelling. These changes were most evident by 1 h, at which time MCHC had decreased by about 0.02–0.04 $g \cdot 100\ mL^{-1}$ at all three temperatures (Table 2). In contrast to the marked reductions in postexercise plasma water content (increased osmolality) and red blood cell swelling, muscle water content appeared to be unaffected by either temperature or exercise. In all three groups of fish the muscle water content fluctuated between 0.70 and 0.75 $L \cdot kg^{-1}$ (Table 2).

Part 2: postexercise survival

No mortality was observed in the control fish (n = 16 per group) at any of the experimental temperatures, or in fish exercised at 12 or 18°C (n = 10 per group). In contrast, 3 of the 10 fish exercised at 23°C died within 4 h, but further mortality was not observed beyond this time. The level of mortality (30%) seen at 23°C was significant (P = 0.046; Fisher's exact test).

Delayed mortality was also observed in the investigation designed to assess postexercise physiology (part 1). At 18°C, one fish of the seven sampled beyond 1 h died following

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Table 2. Changes in plasma osmolality, mean cell haemoglobin concentration (MCHC), and white muscle water content in exhaustively exercised Atlantic salmon at 12, 18, and 23°C.

		Osmolality ($mosmol \cdot kg^{-1}$)	MCHC ($g \cdot 100\ mL^{-1}$)	H_2O content ($L \cdot kg^{-1}$)
12°C	Rest	326±6	0.300±0.01	0.71±0.00
	0 h postexercise	359±7*	0.282±0.01	0.72±0.01
	1 h postexercise	390±9*	0.260±0.00*	0.73±0.01
	4 h postexercise	355±11	0.282±0.01	0.74±0.01
18°C	Rest	323±8	0.288±0.01	0.71±0.02
	0 h postexercise	369±16*	0.255±0.00*†	0.70±0.01
	1 h postexercise	330±8†	0.253±0.00	0.76±0.02
	4 h postexercise	328±3	0.279±0.01	0.74±0.00
23°C	Rest	348±5‡	0.277±0.00	0.73±0.02
	0 h postexercise	381±7*	0.253±0.01†	0.72±0.01
	1 h postexercise	350±6†	0.239±0.01*†	0.72±0.01
	4 h postexercise	337±12	0.263±0.02	0.71±0.03

Note: Values are given as the mean ± 1 SEM (n = 5–8, except after 4 h at 23°C where n = 2).

*Significant difference from resting value ($P < 0.05$) at each respective temperature.

†Significant difference from 12°C fish ($P < 0.05$) for each sampling period.

exercise. At 23°C, three of six fish died between 1.5 and 2.5 h, and a fourth fish died just before the 4-h sampling period.

Discussion

Exhaustive exercise as a model for angling

Several studies have now looked at metabolic fuel depletion and recovery in Atlantic salmon that were angled in the wild (Booth et al. 1995; Brobbel et al. 1996; Wilkie et al. 1996). These studies demonstrate that resultant changes in blood lactate and white muscle glycogen, lactate, pH_i, metabolic proton load, and P_{CO_2} are comparable to changes observed in fish that were exhaustively exercised using well-established methods such as chasing or swim tunnels (e.g., Milligan and Wood 1986*b*; Pearson et al. 1990; Schulte et al. 1992; Kieffer et al. 1994; Wang et al. 1994*a*). Thus, it appears that the exhaustive chasing protocol used in this and previous studies serves as a useful model to describe disturbances that take place in the blood and white muscle during angling in the Atlantic salmon and perhaps other game fish species.

Metabolic fuel depletion and recovery

Temperature had no effect on the resting white muscle ATP or PCr concentrations of Atlantic salmon. The similar ATP levels seen at all three temperatures agree with previous observations indicating that acclimation temperature does not influence resting white muscle ATP concentrations in salmonids (Kieffer et al. 1994). However, the resting concentrations of white muscle ATP and PCr measured here were slightly higher than measurements made on wild, prespawning Atlantic salmon (Booth et al. 1995) and much greater than levels typically measured in rainbow trout (Milligan and Wood 1986*b*; Dobson and Hochachka 1987; Mommsen and Hochachka 1988; Schulte et al. 1992; Wang et al. 1994*a*, 1994*b*). Indeed, high levels of white muscle ATP and PCr might account for the Atlantic salmon's apparently higher capacity for burst exercise (e.g., Tufts et al. 1991).

Although exercise led to large decreases in white muscle ATP and PCr stores, we did not detect a complete depletion of high-energy phosphogens. Several studies have reported near depletions of ATP and PCr immediately following exhaustive exercise in rainbow trout (e.g., Milligan and Wood 1986*b*; Dobson and Hochachka 1987; Mommsen and Hochachka 1988; Kieffer et al. 1994), while others have only observed partial depletions of these fuels (Wang et al. 1994*a*; Wilkie et al. 1996). This variability might be related to the utilization of different exercise protocols (e.g., swim tunnels versus chasing versus angling) or sampling procedures, but it might also be species related. It should be kept in mind, however, that observed decreases in glycogen (see below) in this and previous exercise or angling studies would not take place unless glycolysis was initiated following significant reductions in white muscle ATP and PCr (Moyes and West 1995).

Temperature clearly influenced the rates of metabolic fuel replenishment following exercise. Although rates of PCr restoration seemed to be independent of temperature, white muscle ATP and glycogen replenishment (glyconeogenesis) were much slower in the cooler water. The rapid recovery of PCr agrees with the findings of several studies focusing on postexercise metabolism in salmonid white muscle (Scarabello et al. 1991; Schulte et al. 1992; Wang et al. 1994*a*; Booth et al. 1995; Wilkie et al. 1996). The slower return of ATP and glycogen to resting levels in cooler water was probably associated with diffusive and enzymatic limitations that negatively influenced oxidative processes in the white and red musculature. At lower temperatures the catalytic efficiency of many metabolic enzymes is lowered (Guderley and Blier 1988) and increased cytosolic viscosity may also slow down oxygen delivery and metabolite exchanges between the cytosol and mitochondria (Sidell and Hazel 1987). By extension, oxygen delivery limitations might also explain why postexercise replenishment of ATP and glycogen, which are highly dependent upon oxidative metabolism (Moyes and West 1995), were slower at 12°C. The absence of decreases in white muscle REDOX state (NAD^+/NADH ratio; Table 1), however, suggests that the slower recovery of white muscle physiological status in cooler water was probably not related to oxygen delivery limitations. Had oxygen been limiting, REDOX would have been reduced because of lower rates of mitochondrial NADH oxidation (Katz and Sahlin 1988; Wang et al. 1994*a*).

Lactate metabolism and acid–base balance

In agreement with previous studies on salmonids (Kieffer et al. 1994), resting and peak postexercise white muscle lactate concentrations were not influenced by acclimation temperature. We are unable to explain why resting glycogen was lower in the 12°C controls, but we are confident that resting muscle glycogen concentrations in the remaining 12°C fish tested were comparable with those at 18 and 23°C because initial increases in muscle lactate were comparable at all three temperatures.

The virtual depletion of glycogen observed at all three temperatures in the present study appears to contradict the results of Kieffer et al. (1994), who reported larger drops in white muscle glycogen in warm-acclimated (18°C) versus cold-acclimated (5°C) rainbow trout. The lowest temperature (5°C)

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used by Kieffer et al. (1994), however, was also well below the optimal thermal range of rainbow trout (10–22°C; Elliott 1981). Because the lowest temperature examined here (12°C) was well within the Atlantic salmon's optimal range (6–20°C; Elliott 1981), we suggest that the absence of glycogen depletion reported by Kieffer et al. (1994) was related to the markedly lower water temperatures their cold-acclimated fish were held in.

The rapid catabolism of postexercise white muscle lactate at 18 and 23°C was temporally associated with the recovery of white muscle glycogen stores. This would be expected because lactate is a critical glyconeogenic substrate (see Suarez and Mommsen 1987; Moyes and West 1995 for reviews). At 12 and 18°C, however, the ratio of lactate removal to glycogen resynthesis was about 4:1, well in excess of the expected ratio of 2:1 (e.g., Moyes and West 1995). Much of this missing lactate was probably oxidized within the white muscle (e.g., Bilinski and Jonas 1972; Turner et al. 1983; Milligan and Wood 1986*b*), while a significant proportion may have been lost to the body's extracellular fluid compartments (Wang et al. 1994*a*), which are made up of the plasma and interstitial fluid spaces (Milligan and Wood 1982). The slower catabolism of lactate at 12°C was probably the result of lower oxidation rates and decreased rates of glyconeogenesis, which were related to limitations in catalytic efficiency and diffusive processes (see above). Persistently lowered pH_i in the colder water also likely compounded the aforementioned limitations and further retarded lactate catabolism and glycogen resynthesis (Milligan and Wood 1986*b*).

Resting white muscle pH_i values at all three temperatures were comparable to measurements made in several previous studies (e.g., Milligan and Wood 1986b; Pörtner et al. 1990; Wang et al. 1994*a*, 1994*b*; Booth et al. 1995). The observation that exhaustive exercise led to comparable, ~0.6 unit, decreases in muscle pH_i at all three temperatures was also similar to recent observations made on the rainbow trout (Kieffer et al. 1994).

It is well established that postexercise acidosis results from high rates of respiratory CO_2 generation, lactic acid production, and ATP hydrolysis (see Hochachka and Mommsen 1983; Wood 1991 for reviews). Following exercise, it is essential that this acidosis be sufficiently corrected so that restorative processes, such as glyconeogenesis, can proceed (Milligan and Wood 1986b). One way pH_i correction might occur is through in situ lactate oxidation in the white muscle, which leads to net H^+ consumption (Bilinski and Jonas 1972; Milligan and Wood 1986*b*). The much slower rates of lactate disappearance at 12°C suggest that in situ lactate oxidation was not likely as rapid in these cold-water acclimated salmon, and this may have ultimately led to relatively lower rates of glyconeogenesis. Because glyconeogenesis also leads to the net consumption of H^+ and lactate (e.g., Hochachka and Mommsen 1983; Milligan and Wood 1986*b*; Walsh and Milligan 1989), slower rates of glyconeogenesis probably compounded the relative inhibition of pH_i restoration that was seen in the salmon recovering from exercise at 12°C. Indeed, Pagnotta et al. (1995) recently suggested that glyconeogenesis plays a critical role in postexercise white muscle pH_i restoration.

Interestingly, the results of the present study contrast with those previously described in wild Atlantic salmon angled at ~20°C during the midsummer (Wilkie et al. 1996). Wilkie et al. reported that angling and postangling recovery in warmer waters were associated with a reduced ability to correct pH_i and impaired glyconeogenesis. Taken together, these observations suggest that additional factors, other than temperature, influence the postangling or exercise physiology of Atlantic salmon. For instance, factors such as the degree of freshwater acclimation and nutritional status might significantly influence the salmon's ability to cope with angling-induced physiological disturbances. Indeed, a recent study by Brobbel et al. (1996) demonstrated that the postangling physiological disturbances in freshwater acclimated kelts (black Atlantic salmon), which had been starved for several months following spawning, were less severe than those experienced by bright salmon, which had just entered fresh water from the ocean.

Blood lactate and osmoregulation

As with previous studies on rainbow trout (e.g., Kieffer et al. 1994), resting blood lactate concentrations were independent of temperature in the Atlantic salmon. Unlike the rainbow trout (Kieffer et al. 1994), however, water temperature did not influence the peak lactate load to the blood of Atlantic salmon following exercise. Again, differences in the range of temperatures studied (see above) may explain the apparent discrepancy between Kieffer et al.'s study and the present investigation. Regardless, the delayed lactate peak, seen at all three temperatures after 1 h of recovery, was also consistent with previous studies on other salmonids (e.g., Black 1957; Milligan and Wood 1986*a*, 1986*b*; Tufts et al. 1991; Pagnotta and Milligan 1991; Schulte et al. 1992; Wang et al. 1994*a*). Lower temperature (12°C), however, markedly reduced the salmon's ability to eliminate this extracellular (ECF) lactate load.

There are at least two potential explanations to describe why the clearance of the Atlantic salmon's postexercise ECF lactate load is slower at 12°C. First, evidence has been accumulating recently that suggests that ECF lactate might be taken back up into the white muscle by a presumptive lactate transporter (e.g., Turner et al. 1983; Milligan and Girard 1993; Wang et al. 1994*a*). Hence, slower elimination of lactate at 12°C in the present study might be explained by lower lactate transporter efficiency in cooler water. Second, a number of workers have also suggested that ECF lactate can be oxidized by other tissues, such as the heart, red blood cells, or liver (Bilinski and Jonas 1972; Lanctin et al. 1980; Wood et al. 1990). Thus, lower rates of lactate oxidation in these tissues at 12°C would also slow down lactate removal from the ECF compartment. It is apparent that more work is needed to define how lactate is eliminated from the ECF following exercise and to elucidate how changes in temperature affect these processes.

The slightly higher resting plasma osmolalities and significantly lower MCHC in salmon held at 23°C suggested that acclimation to the higher temperature resulted in some contraction of the ECF compartment and water uptake into the red blood cells, respectively. However, these effects were slight, and the similar resting white muscle water contents in all three groups of fish implies that osmotic balance was not significantly compromised by temperature acclimation to 23°C. Exercise, however, resulted in considerable disruption of osmotic balance. The large increases in plasma osmolality, accompanied by marked reductions in MCHC, suggest that there was significant haemoconcentration following exercise at all three

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temperatures. Notably, the magnitude of the immediate change in osmolality was similar at all three temperatures. Osmotic disturbances in fish are primarily corrected through the modulation of branchial, white muscle and renal ion exchange processes (see Wood 1991 for review). Hence, the markedly slower correction of the osmotic disturbance observed in the salmon at 12°C may have been related to slower turnover rates of branchial, renal, and perhaps white muscle ion transporters. In fact, Gonzalez and McDonald (1994) recently reported that branchial Na^+ uptake and diffusive Na^+ losses were lower in trout acclimated to cold, 6°C, water. By extension, these arguments might also explain why osmolality continued to rise during the 1st h of recovery at 12°C.

Postexercise survival

Exhaustive exercise at 23°C clearly made Atlantic salmon more susceptible to delayed postexercise mortality. These observations are important because wild Atlantic salmon are frequently angled and released during the warm summer months, when temperatures in the rivers and streams of Atlantic Canada may approach 23°C (Caissie 1995). Recently, we also observed 40% mortality in Atlantic salmon that were angled during the midsummer in the Miramichi River, New Brunswick, at ~20°C (Wilkie et al. 1996). In contrast, no mortality was observed in similar studies conducted at 4–6°C in the fall and spring (Booth et al. 1995; Brobbel et al. 1996).

Delayed mortality following angling or exercise has been reported in several gamefish species (e.g., Black 1958; Bouck and Ball 1966; Beggs et al. 1980; Graham et al. 1982; Ferguson and Tufts 1992; Fielder and Johnson 1994), but to date there is no clear explanation of why exhaustive exercise sometimes results in death. Wood et al. (1983) suggested that the magnitude of the white muscle intracellular acidosis might be an important determinant of delayed postexercise mortality. The data presented here, however, suggest that white muscle pH_i is readily corrected at higher temperatures. These observations are not consistent with intracellular acidosis being the causative factor of mortality following exercise. The absence of any reductions in REDOX during the postexercise recovery period also tends to argue against oxygen delivery limitations as a cause of postexercise mortality in the present experiment. It should be noted, however, that the data presented here only represent the physiology of surviving fish, and it is likely that the physiology of dying fish may have been markedly different. Although it would have been desirable to examine separately the physiology of survivors and nonsurvivors in this study, we were unable to do so because metabolite analysis can only be performed upon muscle samples that are taken terminally, following anaesthetization (Tang and Boutilier 1991; Wang et al. 1994*b*). These methodological limitations make it impossible to follow changes in muscle physiology in the same animal over time and, therefore, to separate survivors from nonsurvivors. Thus, further research involving a different methodological approach is still required to determine what the causative factor(s) are in delayed postexercise or angling mortality in fish.

In summary, although warmer water appeared to facilitate the recovery of white muscle ATP, glycogen, lactate, pH_i, and osmotic balance to resting levels in exhaustively exercised Atlantic salmon, it also resulted in elevated postexercise mortality. Clearly, the results of this study, combined with our earlier investigations (Booth et al. 1995; Wilkie et al. 1996; Brobbel et al. 1996), indicate that catch and release angling is more effective at lower water temperatures. In view of these facts, it appears that temporary closures of the recreational fishery for Atlantic salmon might have to be considered as a conservation measure when water temperatures become extremely high (i.e., ≥23°C).

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Acute physiological effects of zebra mussel (*Dreissena polymorpha*) infestation on two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*

S.M. Baker and D.J. Hornbach

Abstract: Our laboratory studies of the physiological effects of zebra mussel (*Dreissena polymorpha*) infestation on the freshwater mussels *Actinonaias ligamentina* and *Amblema plicata* (Unionidae) show that (*i*) zebra mussel infestation causes stress and symptoms of starvation in unionid mussels, (*ii*) unionid species are affected unequally, and (*iii*) symptoms of starvation are greater when initial condition is low. Nutritive stress in infested unionid mussels was indicated by shifts to lower metabolic rates, more protein-based metabolism (lower O:N ratios), and compensatory increases in grazing rates. Starvation may be the result of local food depletion and (or) increased metabolic cost. *Actinonaias ligamentina* (subfamily Lampsilinae) was more sensitive to infestation than *Amblema plicata* (subfamily Ambleminae), as indicated especially by changes in oxygen uptake rate and grazing rate. The effects of infestation were greater in mussels that were already in low condition. Our results indicate that the decline in diversity of unionid mussels since the introduction of zebra mussels is due to species-specific rates of starvation.

Résumé : Nos études de laboratoire sur les effets physiologiques de l'invasion des moules zébrées (*Dreissena polymorpha*) sur les mulettes *Actinonaias ligamentina* et *Amblema plicata* (Unionidae) montrent que (*i*) l'infestation par la moule zébrée cause du stress et des symptômes d'inanition chez les unionidés, (*ii*) les espèces d'unionidés sont affectées de façon inégale et (*iii*) les symptômes d'inanition sont plus prononcés quand l'état de départ est faible. Le stress alimentaire chez les mollusques unionidés affectés se traduit par une baisse des taux métaboliques, un métabolisme davantage basé sur les protéines (rapports O:N plus bas) et une augmentation compensatoire des taux de broutage. L'inanition peut résulter d'une raréfaction locale des ressources alimentaires et (ou) d'un accroissement du coût métabolique. *Actinonaias ligamentina* (sous-famille Lampsilinae) était plus sensible à l'infestation que *Amblema plicata* (sous-famille Ambleminae), ce que révèlent particulièrement les changements dans le taux d'assimilation de l'oxygène et le taux de broutage. Les effets de l'infestation étaient plus manifestes chez les mollusques dont l'état était faible au départ. Nos résultats indiquent que la baisse de la diversité chez les mollusques unionidés depuis l'introduction des moules zébrées est due à des taux d'inanition spécifiques aux espèces.
[Traduit par la Rédaction]

Introduction

Freshwater mussels (family Unionidae) are distributed worldwide and reach their greatest diversity in North America (Williams et al. 1993). These partially infaunal bivalves are ecologically important in both riverine and lotic habitats. As filter feeders, they control seston and act as a trophic link to higher predators (Vannote et al. 1980). Unionid mussels are among the most imperiled groups of animals in the world. Their abundance and diversity have declined dramatically in the last 30 years. There are currently 297 species and subspecies of unionid mussels in North America. Of those, 42 species are federally listed as endangered or threatened, and 69 are being considered for protection. Twenty-one species are presumed extinct (Fish and Wildlife Service 1991; Bogan 1993; Williams et al. 1993). Habitat destruction (including increased siltation, pollution, and river modification, loss of fish hosts, commercial exploitation, and introduced species) are the primary causes of their decline (Bogan 1993). Freshwater mussels live in specific habitat types and are sensitive to many environmental changes.

The recent introduction of the zebra mussel (*Dreissena polymorpha*) to North American freshwater systems poses another threat to already stressed populations of native mussels. Zebra mussel are epifaunal bivalves that colonize all hard substrates, including unionid mussels, by secreting adhesive byssal threads. Having evolved without fouling organisms, unionid mussels have no mechanisms for dealing with epibionts. The decline of unionid mussel populations following the introduction of zebra mussel has been well documented. As early as 1938, Sebestyen suggested that *D. polymorpha* was responsible for the near disappearance of unionid mussels from Lake Balaton, Hungary. A dramatic decline in the abundance of unionid mussels in Lake St. Clair has been observed since the invasion of zebra mussel (Hunter and Bailey 1992; Gillis and Mackie 1994; Nalepa 1994). Schloesser and Nalepa

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(1994) reported 100% mortality of unionid mussels at sites in Lake Erie because of zebra mussel infestation. The relative amount of stress caused by zebra mussel may be species specific. For example, members of the subfamilies Anodontinae and Lampsilinae (long-term brooders) are more sensitive to zebra mussel infestation than are Ambleminae (short-term brooders) (Haag et al. 1993). This suggests that zebra mussel introduction could drastically alter unionid community structure and general biodiversity by affecting the fitness of community members unequally.

Dreissenids have the potential to affect unionid mussels both directly, by actual attachment, and indirectly, through competition for food and nutrients (Hebert et al. 1989; Mackie 1991). In this study, we tested the following hypotheses: (*i*) zebra mussel infestation of unionid mussels causes starvation, (*ii*) unionid mussels in the subfamily Lampsilinae will show greater symptoms of starvation than those in the subfamily Ambleminae, and (*iii*) symptoms of starvation will be greater in unionid mussels that are in low condition. Physiological state was assessed by measuring metabolic rates, O:N ratios, and grazing rates.

Materials and methods

Mussel collection and maintenance

We completed two experiments with each of two species of unionid mussels in fall and spring. In the first two experiments we examined the effects of zebra mussel infestation on *Actinonaias ligamentina*, a long-term brooder in the subfamily Lamsilinae. In the third and fourth experiments, we examined the responses of the short-term brooder, *Amblema plicata* (subfamily Ambleminae).

Specimens of *Actinonaias ligamentina* were collected by SCUBA from the St. Croix River at Wild River State Park, Minnesota, in September 1993 and May 1994. *Amblema plicata* were collected from the St. Croix River at Lakeland, Minnesota, in November 1994 and May 1995. Specimens of *Actinonaias ligamentina* used in the experiments were between 47 and 130 g total wet mass; *Amblema plicata* were between 56 and 315 g. Zebra mussel, *D. polymorpha,* were collected by benthic grab or SCUBA from Lake Michigan at Oak Creek, Wisconsin, in November 1993 and June 1994. Subsequent groups of zebra mussel were collected by SCUBA from Lake Michigan at Milwaukee, Wisconsin, in November 1994 and May 1995. The Milwaukee zebra mussel were larger than the Oak Creek mussel.

To infest experimental unionid mussels with zebra mussel, the unionids were inserted into fine gravel in aquaria, in their natural infaunal position. A layer of zebra mussel was placed on top of them and allowed to attach to the unionids for 48 h. Control unionid mussels were also inserted into gravel in aquaria but were not covered by zebra mussel. Infested specimens of *Actinonaias ligamentina* had an average of 67 ± 3 (mean ± SE) zebra mussel attached, equal to 12.7 ± 0.72 g wet mass. *Actinonaias ligamentina* specimens, therefore, carried 18 ± 1.3% of their own mass (wet), or 14 ± 1.1% by dry mass. Specimens of *Amblema plicata* had an average of 28 ± 1 zebra mussel attached, equal to 28.2 ± 1.0 g wet mass. *Amblema plicata* specimens, therefore, carried 19 ± 0.8% of their own mass (wet), or 19 ± 1.5% by dry mass. The mean numbers of zebra mussel per unionid observed in the field range from less than 10 to over 600 individuals (Lewandowski 1976; Gillis and Mackie 1994). Following infestation, mussels were maintained in Frigid Units Living Streams at 17 ± 1°C, approximating early fall and late spring temperatures in the St. Croix River. The recirculating streams held 500 L of water and had screen and charcoal filtering. Each stream contained 10 cm of substrate, with proportions of cobble, gravel, and sand approximating that of the St. Croix River. Control and infested unionid mussels were held in separate streams.

The mussels were fed one of two diets during an experiment: (*i*) dehydrated *Chlorella* sp. (Algae-Feast, The Earthrise Co., Callpatria, Calif.) (15% lipid, 54% protein, 16% carbohydrate, and 12% inorganic material) or (*ii*) a mixture of preserved diatoms, primarily *Thalassiosira pseudonana* and *Skeletonema* sp. (Diet C, Coast Seafoods, Co., Quilcene, Wash.) (10% lipid, 32% protein, 14% carbohydrate, and 45% inorganic material). *Actinonaias ligamentina* collected in the fall were fed the *Chlorella* diet, while specimens collected in the spring were fed the diatom diet. *Amblema plicata* collected in the fall were fed the diatom diet, while those collected in the spring were fed the *Chlorella* diet. We considered the *Chlorella* diet to be the poorer of the two diets for two reasons. First, bivalves fed diets of *Chlorella* sp., freeze-dried foods, or unialgal cultures grow poorly (Walne 1970; Millican and Helm 1994). Second, we observed that the dried *Chlorella* diet tended to form clumps and quickly settled out of the water column, making it unavailable to the mussels. Therefore, mussels on the diatom diet were fed approximately 1.5% of their dry tissue mass per day, while mussels on the *Chlorella* diet were fed more, at approximately 2.2% of their dry tissue mass per day.

Physiological measurements

In the first experiment, with *Actinonaias ligamentina* collected in the fall, physiological measurements were made at 1, 2, and 3 months following initial infestation. In subsequent experiments, physiological measurements were made at 1, 2, and 4 months following initial infestation. Oxygen uptake, ammonia excretion, and grazing rates were measured for 10–15 control and 10–15 infested unionid mussels, from which zebra mussel had been carefully removed. Water from the streams was filtered (0.45 μm) for use in the experiments. Metabolic chambers consisted of acrylic containers with total volumes of 1400 mL. Temperature was maintained at 18°C (similar to holding temperature) by immersing the chambers in a temperature controlled water bath containing submersible magnetic stirrers. Water in the metabolic chambers was stirred with magnetic bars throughout all experiments. Mussels were scrubbed before experiments and individuals were placed on grid platforms in the chambers.

Rates of oxygen uptake by individual unionids were measured as the oxygen depletion in the metabolic chambers. Oxygen concentrations were measured with YSI oxygen probes (5331) held in Lucite plungers, inserted into the metabolic chambers and connected to YSI biological oxygen monitors (5300). The oxygen probes were calibrated daily with air-saturated water (100% of air saturation). Ten metabolic chambers and two control chambers, without mussels, were run concurrently. Oxygen concentration in each chamber was recorded every 30 min for up to 4 h. Measurements were stopped if the oxygen concentration in a chamber decreased to 50% of air saturation. Although we did not determine critical oxygen concentrations for these species, our data indicated that above oxygen concentrations of 50% of air saturation, metabolic rates were independent of oxygen availability. Air saturation values reached average lows of 75 and 69% in the *Actinonaias ligamentina* and *Amblema plicata* experiments, respectively. Oxygen uptake rates were corrected for the volume of water displaced by the mussels, barometric pressure, and any changes in oxygen concentration in the control chambers.

Nitrogen excretion, measured as the accumulation of ammonia and urea during the oxygen uptake experiments, was measured using an Orion ammonia electrode (model 95-12) connected to an Orion bench-top pH–ISE meter (model 920A). Two 50-mL water samples were drawn from the metabolic chambers immediately upon ending the oxygen uptake measurements. Any urea in the samples was converted to ammonia by adding 3 mg of urease powder (Fisher Scientific, Fair Lawn, N.J.), according to Orion Research Inc. (1990). Samples were stirred during measurements. Four standard curves were produced each day by adding known quantities of Orion ammonia standard (ammonia chloride, 1000 ppm as N) to samples from the control chambers. Ammonia excretion rates were calculated using the

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standard curves and corrected for the ammonia concentrations in the control chambers. Atomic ratios of O:N were calculated from the oxygen uptake and nitrogen excretion rates. O:N ratios have been used as a measure of physiological stress (Bayne and Widdows 1978; Widdows 1978).

Grazing rates of individual unionid mussels, measured as the decrease in light absorbance of an algal suspension, were obtained with a Brinkman probe colorimeter (PC 800) equipped with a 450-nm filter, fiber optic light guide, and stainless steel probe tip (5-cm light path) (see Hornbach et al. 1991). The colorimeter was calibrated before each use with distilled water (0% absorbance). Individual unionid mussels were placed in the metabolic chambers in 1000 mL of water. Ten metabolic chambers and one control chamber were run concurrently. Diatom diet was added to the chambers to an initial concentration of 11.6 ± 1.2 mg dry mass·L^{-1} (6.4 mg ash-free dry mass). This concentration approximates the mean total suspended solids observed in the St. Croix River (D.J. Hornbach, unpublished data; U.S. Geological Survey data retrieved through STORET). Light absorbance of each chamber was recorded every 15 min for 90 min. A standard curve was produced each day. Known quantities of diatom diet were added to six 1-L volumes of water, the absorbance was measured, and the water was filtered onto preweighed Whatman glass microfiber filters (934-AH). The filters were then dried (100°C for 24 h) and weighed. Grazing rates were calculated according to Coughlan (1969) and Sprung (1984) using the standard curve and corrected for absorbance changes in the control chamber.

Unionid mussels were wet weighed and measured for height, length, and width. Upon completion of the monthly physiological measurements, the tissues were removed from the shells and the presence or absence of brooded glochidia in the gills was noted. Visceral dry mass of each mussel was determined by oven drying (100°C for 24 h) or by freeze drying to constant mass. Freeze-dried tissues did not lose additional mass if they were oven dried. Unionid mussel shells were dried for several days at room temperature and weighed.

Condition index, a commonly used measure of nutritive status in marine bivalves, was calculated according to Crosby and Gale (1990): condition index = (dry tissue mass (g) × 1000)/internal shell cavity capacity (g), where internal shell cavity capacity (g) = total wet mass (g) – dry shell mass (g).

Data distributions were tested for normality using Shapiro–Wilk *W* tests and visually inspected using normal quantile plots. Condition index and O:N ratios were arcsin transformed (Zar 1984). Oxygen uptake, ammonia excretion, grazing rates, and dry mass were log transformed (Zar 1984). Analyses of covariance were performed to test the null hypotheses that there were no effects of treatment (infested versus control), collection season (fall versus spring), or interaction on the physiological measures of condition index, oxygen uptake, ammonia excretion, O:N ratio, or grazing rate. Dry mass per individual and days of infestation (30, 60, 90 or 120) were the covariables. If interactions were significant, Student–Newman–Keuls (S–N–K) multiple comparison tests were used to identify differences between specific treatment and season combinations. For months in which sufficient brooding mussels were found, an analysis of covariance (dry mass per individual as covariable) was conducted to test the null hypothesis that there was no effect of reproductive state on the physiological measures. Data are reported as the back-transformed least squares means and standard errors of the means. Statistical analyses were conducted using JMP version 3 software (SAS Institute Inc. 1994).

Results

For both *Actinonaias ligamentina* and *Amblema plicata*, fall-collected specimens had lower condition indices than did spring-collected specimens ($F_{[1,134]} = 54.9$, $P \leq 0.0001$; $F_{[1,166]} = 6.8$, $P = 0.01$) (Fig. 1A). *Actinonaias ligamentina* had a lower condition index than *Amblema plicata* (Fig. 1A). Mass-specific rates of oxygen uptake, ammonia excretion, and grazing were higher in *Actinonaias ligamentina* than in *Amblema plicata* (Figs. 1B–1E). The condition index of *Actinonaias ligamentina* did not change ($F_{[1,134]} = 1.3$, $P = 0.26$) over the course of the experiment regardless of treatment, collection season, or diet. In *Amblema plicata*, condition index decreased (20%) over the course of the experiment ($F_{[1,166]} = 46.9$, $P \leq 0.0001$); however, the decline was similar regardless of treatment, collection season, or diet.

For *Actinonaias ligamentina*, there were significant interactions between treatment and collection season in rates of ammonia excretion ($F_{[1,133]} = 5.0$, $P = 0.03$) and grazing ($F_{[1,132]} = 7.1$, $P = 0.009$) (Figs. 1C and 1E). Unionids infested by zebra mussel or collected in the fall had lower ammonia excretion rates (S–N–K, $P \leq 0.0001$) than controls collected in the spring (Fig. 1C). Mussels collected in the fall and infested by zebra mussel had greater grazing rates (S–N–K, $P \leq 0.0001$) than did the other three treatment–season combinations (Fig. 1E). Both treatment and collection season had significant effects on the oxygen uptake rates of *Actinonaias ligamentina* (Fig. 1B). Unionids infested with zebra mussel had lower oxygen uptake rates ($F_{[1,129]} = 14.7$, $P = 0.0002$) than control mussels. Oxygen uptake was lower ($F_{[1,129]} = 20.9$, $P \leq 0.0001$) in mussels collected in the fall than in those collected in the spring. Only O:N ratios were not significantly affected by treatment, collection season, or their interaction (Fig. 1D).

Two of 10 control specimens and 1 of 10 infested specimens of *Actinonaias ligamentina* were brooding glochidia during the day 30 measurements of the fall experiment. One of 10 control specimens and 5 of 9 infested specimens were brooding during the day 60 measurements of the fall experiment. The number of infested specimens brooding in the day 60 measurements was sufficient to test the effects of brooding on physiology. Brooding had no effect on condition index, oxygen uptake, ammonia excretion, O:N ratio, or grazing rate.

No specimens of *Amblema plicata* were found to be brooding glochidia during the experiments. For *Amblema plicata*, there was a significant interaction between treatment and collection season in rate of oxygen uptake ($F_{[1,171]} = 12.5$, $P = 0.0005$) (Fig. 1B). Oxygen uptake was lower (S–N–K, $P = 0.008$) in infested mussels and those collected in the fall than in control mussels collected in the spring. The interaction between treatment and collection season was nearly significant for O:N ratios ($F_{[1,164]} = 3.7$, $P = 0.06$) (Fig. 1D); mussels infested by zebra mussel and (or) collected in the spring had lower O:N ratios than control mussels collected in the fall. Treatment also had a significant effect on the grazing rates of *Amblema plicata* (Fig 1E). Unionids infested with zebra mussel had greater grazing rates ($F_{[1,168]} = 5.9$, $P = 0.02$) than control mussels. Collection season also had a significant effect on ammonia excretion rates (Fig 1C). Ammonia excretion rates were lower ($F_{[1,173]} = 26.5$, $P \leq 0.0001$) in mussels collected in the fall than in those collected in the spring.

Discussion

Our study shows that (*i*) zebra mussel infestation causes stress and symptoms of starvation in unionid mussels, (*ii*) unionid

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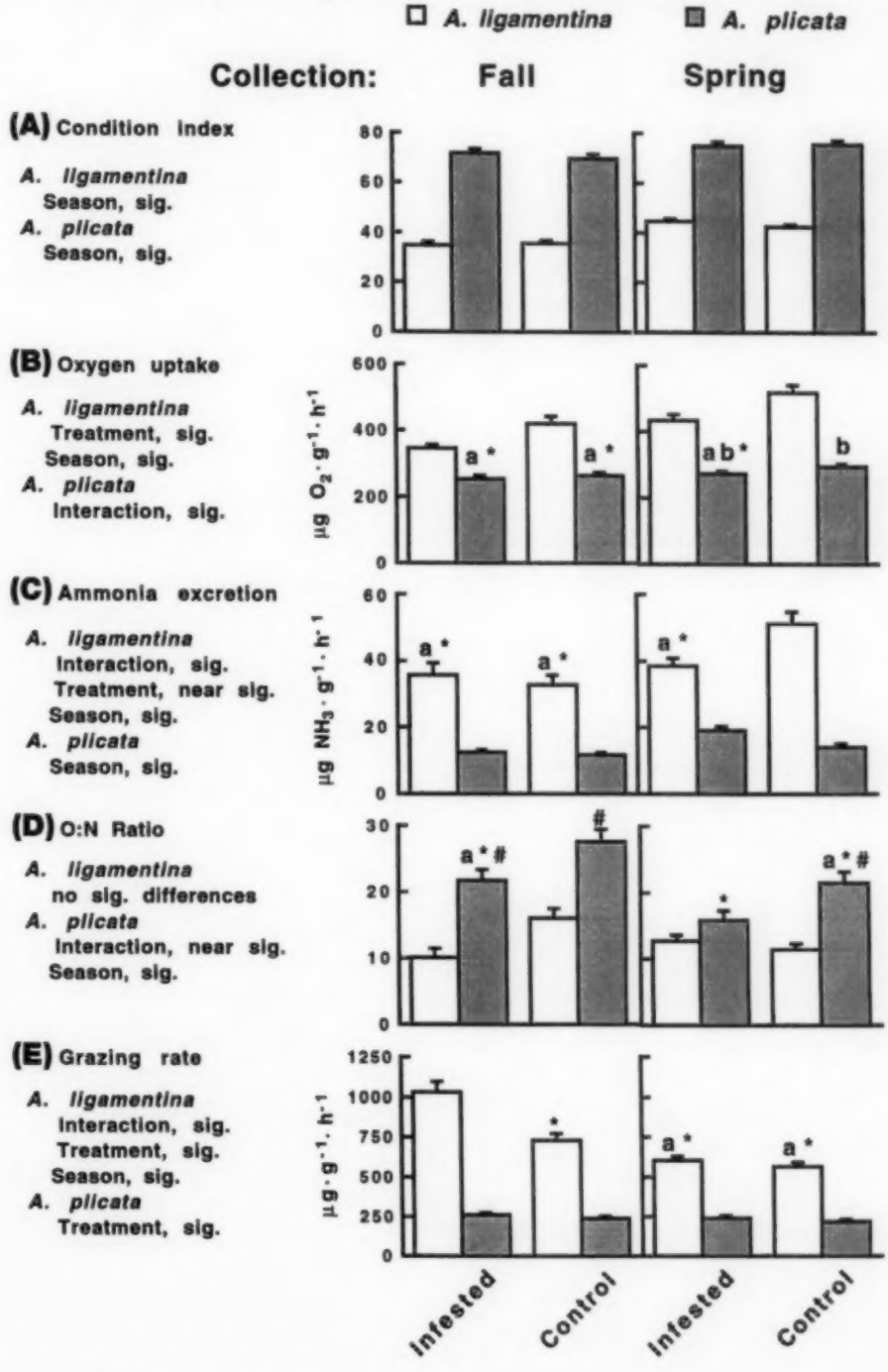
Fig. 1. *Actinonaias ligamentina* (open columns) and *Amblema plicata* (filled columns): (A) condition index, (B) oxygen uptake rates, (C) ammonia excretion rates, (D) O:N ratio, and (E) grazing rates of control mussels and mussels infested by the zebra mussel, *Dreissena polymorpha*. Mussels were collected in either the fall or spring. Sig., significant differences between treatments (control or infested), collection seasons (fall or spring), or their interaction. For those parameters in which there was a signficant interaction between treatment and collection season, columns that are not significantly different are indicated. Letters (*a* or *b*) indicate pairs of columns within a species that are not significantly different. Symbols (* or #) indicate triplets of columns within a species that are not significantly different (mean ± SE; some SEs are too small to be shown, n = 131–136 for *Actinonaias ligamentina*, n = 166–175 for *Amblema plicata*).



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mussel species are affected unequally, and (*iii*) symptoms of starvation are greater when initial condition is low. Of the two mussels examined, *Actinonaias ligamentina* was the most sensitive to infestation as indicated especially by changes in oxygen uptake rate and grazing rate. This supports the report by Haag et al. (1993) that mussels in the subfamily Lampsilinae are more affected by zebra mussel infestation than are those in the subfamily Ambleminae. Following infestation, both species had lower metabolism and greater grazing rates.

Several mechanisms have been suggested that may reduce fitness of unionids colonized by zebra mussels, eventually resulting in mortality. Researchers have proposed that zebra mussel infestation may (*i*) impair locomotion, burrowing, and balance; (*ii*) interfere with valve closure, increasing the risk of predation and parasitism; (*iii*) hinder valve opening, impairing feeding, reproduction, respiration, and excretion; (*iv*) smother siphons, obstructing respiration and feeding; (*v*) strip inhalant water of food, reducing food intake; (*vi*) cause shell deformities, interfering with normal growth; and (*vii*) generate toxic metabolic wastes (Schloesser and Kovalak 1991; Mackie 1991). Our physiological studies indicate that starvation, owing to reduced food intake and (or) increased metabolic costs, is the major mechanism that reduces fitness and eventually causes mortality of unionid mussels infested with zebra mussel.

Ricciardi et al. (1996) reported a decline in unionid mussel condition corresponding with an increase in zebra mussel infestation in the St. Lawrence River. Condition index is proportional to energy stores and is therefore an indication of the health of a bivalve (Bayne et al. 1985). Reductions in the condition of bivalves have been reported as the result of parasitism (Barber et al. 1988), competition for food and space (Côté et al. 1994), turbidity (Mason and Nell 1995), and exposure to metals (Naimo et al. 1992; Couillard et al. 1995). In our short-term experiments, we found that zebra mussel infestation did not affect the condition index of either *Actinonaias ligamentina* or *Amblema plicata*. However, in both species, condition index was higher if mussels were collected in the spring, regardless of the diet subsequently fed to them. This indicates that in these two species of unionid mussel, energy stores are greater in the late spring than they are in the fall.

The condition index of *Actinonaias ligamentina* did not change during the experiments, regardless of which diet was fed to them. This suggests that the *Chlorella* diet and the diatom diet provide equally adequate nutrition for this species. The condition index of *Amblema plicata*, however, decreased during the experiments, but the decrease was the same with both diets. This indicates, again, that the two diets are equally nutritious but that we did not feed *Amblema plicata* enough of either diet.

Metabolic rates of bivalves are influenced by stress and starvation. Stress caused by parasitism (Bierbaum and Shumway 1988; Holopainen and Penttinen 1993) or exposure to toxic metals (Naimo et al. 1992) results in decreased metabolic rates. Starvation or poor diets also lower the metabolism of both freshwater and marine bivalves (Lomte and Nagabhushanam 1971; Bayne 1973; Grant and Cranford 1991). We found that, either alone or in interaction with initial condition, zebra mussel infestation significantly reduced the oxygen uptake rates of both of the mussel species that we examined. In our study, zebra mussels were removed prior to oxygen uptake measurements, so the decline in oxygen uptake rate was not an instantaneous response to infestation. Instead, the reduction in oxygen uptake clearly shows a degradation in nutritional status and a shift to a conservative metabolic strategy. Specimens of *Amblema plicata* that were already in poor condition (collected in the fall) may have been metabolizing near their basal rate and unable to decrease their metabolism further. Oxygen uptake rates indicate that zebra mussel infestation causes stress and (or) starvation, that *Actinonaias ligamentina* is more sensitive to infestation than *Amblema plicata*, and that the degree of response to zebra mussel can depend on the initial condition of the unionid mussel.

Bivalves exhibit a variety of shifts in protein turnover and thus ammonia excretion rates in response to stress and starvation. Marine bivalves increase protein degradation and thus excretion rates in response to stress (Grant and Thorpe 1991), although the initial condition of the bivalve may influence this response. Bayne (1973) found that marine mussels collected in summer (high carbohydrate reserves) decreased ammonia excretion rates in response to nutritive stress whereas mussels collected in winter (low carbohydrates) increased ammonia excretion. Freshwater mussels lower their excretion rates in response to stressors such as metals and turbulence (Aldridge et al. 1987; Naimo et al. 1992). We observed a species-specific response to zebra mussel infestation in the rates of ammonia excretion by unionid mussels. Ammonia excretion rates again indicate that *Actinonaias ligamentina* is more sensitive to zebra mussel infestation than *Amblema plicata*. The variations in response to infestation may reflect species-specific and (or) seasonal differences in tissue chemical composition. The relationships between protein intake, synthesis, catabolism, and deposition in bivalves are not clearly understood.

Atomic O:N ratios provide a more accurate index of metabolic substrate than ammonia excretion alone. O:N ratios are a measure of the balance between the breakdown of proteins and the catabolism of carbohydrates and lipids. Carbohydrate, specifically glycogen, is the major energy storage material in bivalve molluscs (Gabbott 1975). Low O:N ratios (<30) in marine bivalves reflect a high reliance on protein catabolism and are associated with severe stress and starvation (Bayne and Widdows 1978; Widdows 1978). Freshwater mussels, however, appear to have low O:N ratios, ranging from 13 to 78, even under favorable conditions (Aldridge et al. 1987; Fujikura et al. 1988; Naimo et al. 1992). These low O:N ratios are attributed to the breakdown of dietary rather than body protein and may be the result of low rates of protein deposition and growth. The unionid mussels that we studied also had low O:N ratios. Control *Actinonaias ligamentina* had an average O:N ratio of 14, while control *Amblema plicata* had an average O:N ratio of 27. These ratios are similar to those for mussels in which measurements were made immediately upon collection from the field (S.M. Baker and D.J. Hornbach, unpublished data).

As with ammonia excretion rates, bivalves exhibit a variety of shifts in O:N ratios in response to stressors. Many marine bivalves show decreases in O:N ratios when stressed or starved, which are attributed to catabolization of stored protein (Widdows 1978; Grant and Thorpe 1991). The effects of stress on O:N may depend on initial levels of reserves, however. Marine mussels collected in winter or spring (low carbohydrate reserves) decrease O:N ratios when nutritively stressed,

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while mussels collected in summer or fall (high carbohydrate reserves) increase the O:N ratio in response to stress (Bayne 1973). The mussels collected in winter or spring begin using stored proteins whereas those collected in summer or fall use stored carbohydrates. Other studies, however, show that whatever the initial level of reserves, starvation results in a rapid reduction in carbohydrates and lipids (high O:N) followed by a balanced use of all body components (lower O:N) (Ansell and Sivadas 1973). In contrast to marine bivalves, freshwater bivalves increase O:N ratios when stressed or starved (Aldridge et al 1987; Naimo et al. 1992). These increases in O:N ratio are attributed to a shift in catabolism to body stores other than protein, such as carbohydrates and lipids. We observed that specimens of *Actinonaias ligamentina* collected in the fall and infested with zebra mussel tended to have lower O:N ratios than control mussels, while those collected in the spring when condition was higher did not. The lower O:N ratios in fall-collected infested *Actinonaias ligamentina* were primarily the result of a relative decrease in oxygen uptake. In *Amblema plicata*, O:N ratios tended to be lower in specimens infested by zebra mussel and were significantly lower in specimens collected in the spring. The low O:N ratios in *Amblema plicata* were primarily the result of relative increases in ammonia excretion. Our O:N data indicate that zebra mussel infestation causes unionids to shift to a more protein-based metabolism, suggesting that food is limiting and (or) metabolic costs are increased, under infestation conditions. The effects of zebra mussel infestation on O:N ratios are species specific, depending on initial condition in *Actinonaias ligamentina*.

Both marine and freshwater bivalves decrease food clearance rates when they are stressed by parasitism (Mane 1975; Bierbaum and Shumway 1988), pollution (Bayne et al. 1979), or high sediment or seston load (Widdows et al. 1979; Aldridge et al. 1987; Grant and Thorpe 1991). Marine bivalves subsisting on a suboptimal diet, however, compensate by increasing filtration rates (Bayne et al. 1993; Iglesias et al. 1992), and starving bivalves boost filtration rates when food is finally supplied (Thompson and Bayne 1972; Bayne et al. 1973; Widdows 1973). Information on the grazing rate response of freshwater mussels to starvation is lacking. In the present study, grazing rates of infested mussels of both species were significantly greater than those of controls. In *Actinonaias ligamentina*, grazing rates were significantly greater in infested mussels collected in the fall when condition was low. In *Amblema plicata*, grazing rates were significantly greater in infested mussels, and fall grazing rates tended to be greater than spring grazing rates. It appears that the infested unionid mussels in our study, especially those in low condition, were experiencing nutritive stress, and when provided with food in the feeding experiments, they compensated by increasing their grazing to above normal rates.

Nutritive stress in infested unionid mussels was indicated by shifts to lower metabolic rates, more protein-based metabolism, and compensatory increases in grazing rates. Several mechanisms working simultaneously may have caused the observed symptoms of starvation. Zebra mussel infestation may cause starvation by increasing the metabolic costs of balance, valve movement, respiration, and excretion. Further, zebra mussel infestation may decrease energy intake as a result of food removal from the inhalant current of the unionid mussel. Zebra mussel attached to individual *Actinonaias ligamentina* and *Amblema plicata* filtered an average of 121 and 130 $mL \cdot h^{-1}$ (approximately 7 and 2 mg dry algae·g dry $mass^{-1} \cdot h^{-1}$), respectively (S.M. Baker and D.J. Hornbach, unpublished data). Specimens of *Actinonaias ligamentina* and *Amblema plicata* filtered an average of 105 and 74 $mL \cdot h^{-1}$ (approximately 0.8 and 0.3 mg dry algae·g dry $mass^{-1} \cdot h^{-1}$), respectively. Therefore, inhalant water could have been turned over 1.1–1.8 times by encrusting zebra mussel before entering the unionid mussel, effectively removing all seston. In addition, Silverman et al. (1995) suggested that zebra mussel are more efficient filter feeders than unionids. Even if food availability was in excess in the water column, food could be limiting in the benthic boundary layer and a depleted feeding zone could develop around the unionid mussel (Wildish and Kristmanson 1984; Fréchette and Bourget 1985; Muschenheim and Newell 1992). Our study indicates that starvation, whether the result of local food depletion or increased metabolic costs, is the underlying cause of the reported decline in energy stores and fitness of infested unionid mussels (Hebert et al. 1991; Haag et al. 1993; Ricciardi et al. 1996) and of the mortalities observed in the Great Lakes (Hunter and Bailey 1992; Gillis and Mackie 1994; Nalepa 1994; Schloesser and Nalepa 1994).

We observed symptoms of starvation despite the short experiment duration (≤4 months) and low infestation load, compared with loads reported for unionid mussels at sites in Lake St. Clair (Mackie 1991; Gillis and Mackie 1994) and western Lake Erie (Schloesser and Kovalak 1991; Schloesser and Nalepa 1994). The infestation loads in this study were similar to those observed in European lakes (Lewandowski 1976), Lake St. Clair sites (Hebert et al. 1989), the upper Mississippi River system (Tucker et al. 1993), and the St. Lawrence River (Ricciardi et al. 1995). Declines in unionid mussel populations have been associated with mean infestations of as few as 10 zebra mussel per unionid mussel (Ricciardi et al. 1996). Even low infestation rates can cause starvation and eventual mortality.

Populations of unionid mussels have suffered species-specific mortality, and thus a decline in diversity, because of zebra mussel infestation. Shell shape and (or) reproductive strategy may be a factor in mortality. Haag et al. (1993) found that, in Lake Erie, there has been a greater reduction in unionid mussels belonging to the subfamilies Anodontinae and Lampsilinae than in those belonging to the subfamily Ambleminae. The Anodontinae and Lampsilinae have light shells and brood their larvae for long periods of time, while the Ambleminae are heavy shelled and brood for shorter periods of time. Thin-shelled, flattened species such as *Potamilus alatus* and *Leptodea fragilis* (both subfamily Lampsilinae) have declined by over 93% in Lake St. Clair. The thicker shelled *Elliptio dilatata* (subfamily Ambleminae) has declined by only 25% since the introduction of the zebra mussel (Gillis and Mackie 1994).

Unionid mussel mortality following infestation is generally attributed to a decline in physiological fitness. Laboratory-infested *Lampsilis radiata* (subfamily Lampsilinae) and *Amblema plicata* have significantly lower energy stores (glycogen content) and greater stress levels (lower inherent cellulase activity) than unionid mussels from which zebra mussel have been removed 4 months previously (Haag et al. 1993). Field-collected specimens of *Lampsilis radiata* also

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have reduced glycogen stores and cellulase activity, while field-collected *Amblema plicata* show no reduction in biochemical fitness (Haag et al. 1993). Hebert et al. (1991) reported that the lipid contents of heavily infested unionid mussels are 50% that of uninfested mussels. To date, little else is known about how zebra mussel infestation affects the physiology of unionid mussels.

In summary, we have shown that zebra mussel infestation of unionids causes stress because of starvation and that stress is greater when the condition of the mussels is already low. We also found that unionids in different subfamilies are affected by zebra mussel infestation to varying degrees and that the decline in diversity of unionid mussels since the introduction of zebra mussel may be due to species-specific rates of starvation. The diversity and geographic distribution of unionid mussels will continue to decrease as areas are invaded by zebra mussel. We predict that zebra mussel related mortalities of unionids will be greater in regions where unionids are already stressed by other environmental or anthropogenic factors. Even in bodies of water where zebra mussel infestation is not great enough to be lethal, there may be long-term changes in population density and diversity because of species-specific metabolic stress leading to reduced fecundity.

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Seasonal changes in habitat use and preference by juvenile brown trout, *Salmo trutta*, in a northern boreal river

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Abstract: By means of electrofishing, we examined seasonal and size-class variation in habitat preference by juvenile brown trout (*Salmo trutta*) in a third-order river in northern Finland. Larger trout preferred deeper stream areas than young-of-the-year fish. At the onset of winter, all trout size-classes moved into shallower water, but this mainly reflected seasonal variation in habitat availability. In winter, trout preferred slowly flowing stream areas, whereas in other seasons the mean water velocities used by trout parallelled habitat availability. In summer and autumn, age-0 fish favoured stream areas with large amounts of aquatic vegetation to provide cover. The largest trout (16–22 cm) occupied habitats with little cover throughout the year, and in winter, all trout avoided areas with high instream cover. In summer, all size-classes preferred small substrates, whereas in winter, areas with cobble–boulder substrates were preferred, especially by trout larger than 10 cm. Wintering trout often shelter among the interstitial spaces of coarse substrates, and to facilitate the survival of juvenile trout through winter, stream management programmes need to ensure that such particles are abundantly available in trout wintering areas.

Résumé : À l'aide de la pêche électrique, nous avons étudié les variations liées à la saison et à la classe de taille dans la préférence concernant l'habitat chez des truites brunes (*Salmo trutta*) juvéniles dans une rivière de troisième ordre du nord de la Finlande. Par rapport aux jeunes de l'année, les truites de grande taille préféraient les zones plus profondes. Au début de l'hiver, toutes les classes de taille se déplaçaient vers des eaux moins profondes, mais cela correspondait globalement à des variations saisonnières de la disponibilité des habitats. En hiver, les truites préféraient les zones à courant lent, tandis qu'aux autres saisons, les vitesses moyennes du courant où se tenaient les truites correspondaient à la disponibilité de l'habitat. En été et en automne, les poissons d'âge 0 recherchaient les zones où la végétation aquatique était abondante et leur assurait un couvert. Les truites les plus grandes (16–22 cm) occupaient les habitats présentant peu de couvert tout au long de l'année, mais en hiver toutes les truites évitaient les zones où le couvert immergé était important. En été, toutes les classes préféraient les substrats de petite taille, tandis qu'en hiver les substrats favoris allaient du galet au bloc, spécialement chez les truites de plus de 10 cm. Les truites qui hivernent s'abritent souvent dans les espaces interstitiels des substrats grossiers, et, pour faciliter la survie des truites juvéniles pendant l'hiver, les programmes de gestion des cours d'eau doivent veiller à ce que ce type de substrat soit abondant dans les zones d'hivernage.
[Traduit par la Rédaction]

Introduction

Numerous studies have examined the habitat requirements of stream fishes to determine the optimal ranges of habitat variables for different fish species and their life stages. Unfortunately, these have often been "snapshot" studies attempting to reveal universal patterns of microhabitat selection on the basis of data collected from only one stream during one season (Gore and Nestler 1988). Seasonal changes in fish habitat use in relation to habitat availability have received less attention among stream biologists (but see Heggenes and Saltveit 1989; Baltz et al. 1991; Heggenes et al. 1991). Yet, profound changes are known to occur in the activity and habitat use of salmonid fishes at the onset of winter (Rimmer et al. 1983, 1984; Cunjak and Power 1986; Heggenes et al. 1993; Riehle and Griffith 1993). It is thus important that habitat preferences of stream fishes be assessed separately for different times of the year, especially in such strongly seasonal environments as boreal streams and rivers (e.g., Heggenes 1994).

Habitat selection may also vary with the size of the fish. Ontogenetic niche shifts often have far-reaching effects on interspecific interactions in lentic fish communities (e.g., Werner and Gilliam 1984; Persson 1991). Salmonids in streams have also been shown to undergo marked ontogenetic habitat shifts, with younger fish generally occupying shallow, slow-flowing stream areas (Bohlin 1977; Moyle and Baltz 1985; Gatz et al. 1987; Greenberg et al. 1994). The "bigger fish – deeper habitat" relationship seems to hold for stream

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fishes in general, and the mechanistic explanation often given to this pattern is the need of prey fish to balance predation risks from piscivorous and wading or diving predators (Power 1987; Schlosser 1987; Harvey and Stewart 1991). Another mechanism potentially contributing to habitat shifts in salmonids is intercohort competition, whereby larger, dominant individuals exclude smaller conspecifics from the energetically most favourable stream positions (Bohlin 1977; Fausch 1984; Hughes and Dill 1990).

In this study, we examined the microhabitat use of brown trout in relation to habitat availability in a third-order river in northern Finland. We developed habitat preference curves for all three size-classes in three seasons (summer, autumn, and winter) using four habitat variables: depth, water velocity, substrate size, and instream vegetation. We also used multivariate approaches to detect seasonal and size-related changes in trout habitat preference.

Study area

The study was conducted in the River Kuusinkijoki, a third-order tributary of the River Oulankajoki in northeastern Finland. The river rises in Lake Alavuotunkijärvi, flows through spruce and pine forests (drainage area of 1006 km^2), and discharges into Lake Paanajärvi on the Russian side of the border. The flow regime is characterized by wide seasonal fluctuations, with peak flows (up to 65 $m^3 \cdot s^{-1}$) occurring soon after snowmelt in May–June. The mean annual discharge is about 10 $m^3 \cdot s^{-1}$, and the lowest flows (about 1–3 $m^3 \cdot s^{-1}$) occur during winter. Water temperature falls below 1°C in late October, and most of the river remains ice covered from mid-November to late April. The river is relatively rich in nutrients (total nitrogen 210–540 $mg \cdot L^{-1}$, total phosphorus 8.0–19.0 $mg \cdot L^{-1}$). Water is circumneutral, with pH of 6.8–7.8 and conductivity of 3.0–7.4 $mS \cdot cm^{-1}$. Kuusinkijoki is a mesohumic river with relatively high water colour (range 30–80 mg $Pt \cdot L^{-1}$).

Our study site, the Raatekoski rapids on the lower courses of the river (66°17′N, 29°54′E), is a 600 m long and 60 m wide riffle section, where fishing is allowed only for research purposes. The population density of juvenile brown trout (*Salmo trutta*) (mean 0.75 fish/m^2) is considerably higher than in other parts of the Oulankajoki river system (Huusko and Korhonen 1993). The upper section of the study site is mainly fast-flowing, shallow riffles, whereas the downstream section is characterized by alternating pool–riffle sequences; the stream gradient in both sections is 0.023. The stream bed is dominated by small boulders and cobbles, interspersed with coarse gravel (Table 1). Submerged vegetation, covering about 40% of the stream bed, is mainly pond crowfoot *Ranunculus peltatus* and aquatic mosses (mainly *Fontinalis* spp.). Brown trout and Arctic bullhead (*Cottus poecilopus*) are the dominant fish species in the study site. European grayling (*Thymallus thymallus*) and European minnow (*Phoxinus phoxinus*) also occur in the study reach, especially in the slow-flowing pool sections.

Materials and methods

Differences in the methods used for locating fish are often considered to be a source of bias in studies of fish microhabitat use (Shirvell and Dungey 1983; Moyle and Baltz 1985; Heggenes et al. 1990). Heggenes et al. (1990) compared three methods (electrofishing, underwater observation, and surface observation) for studies of habitat use by brown trout and Atlantic salmon (*Salmo salar*). They concluded that no method works well under all conditions. Diving and surface observation yielded accurate results in slow-flowing habitats with fine substrate and without surface turbulence. Electrofishing was found to be the most effective method in shallow, fast-flowing habitats with coarse substrates. Since our study site was dominated by relatively shallow, moderate- to fast-flowing riffles, and the substrate was mainly coarse gravel, cobbles, and boulders, we chose to use electrofishing as the method of fish sampling. Moreover, our study included sampling during winter, and Heggenes and Saltveit (1989) have recently shown that because young salmonids tend to hide in the substrate at low temperatures, underwater observation is not an effective method for studying habitat selection by trout in winter. On one occasion (July 1992) underwater observations were used for locating fish in deeper stream areas (>50 cm) using the methods described by Heggenes and Saltveit (1989), but low water visibility (<3 m) prevented this method from being used on later occasions. We emphasize that although electrofishing seems to be the best alternative for locating fish in humic streams, our data on trout microhabitats likely represent a combination of feeding and refuge sites (see Gatz et al. 1987; Heggenes 1988).

Fish were sampled by two people, one operating the shocker, the other using a dip net to catch stunned fish. To reduce the effects of positive galvanotaxis induced by DC electroshockers (Gatz et al. 1987; Heggenes et al. 1990) we used a DC backpack electroshocker with low voltage (350 V) and a 9 cm diameter anode. Copp (1989) showed that the approximate area of the effective field of a 10-cm anode at each point sample is 0.071 m^2. To minimize the fright bias, which may cause displacement of individuals from their original positions, we used a modified point electrofishing procedure (e.g., Moyle and Baltz 1985; Heggenes 1988). The activated anode was submersed for 5 s at equidistant points (every 0.5 m) along cross-sectional transects spaced 1 m apart along the stream section to be sampled. The point of the first sighting of a fish was marked with a numbered float anchored to the stream bed. Trout captured were measured and assigned to one of three size-classes (4–9, 10–15, 16–22 cm), roughly corresponding to fish ages of 0+, 1+, and 2+, respectively (A. Huusko, unpublished data; see also Belaud et al. 1989).

Habitat use by brown trout was studied in four seasons: midsummer (mid-July 1992), late summer (mid-August 1992), autumn (September – early October 1993), and winter (early April 1994). Six separate stream sections, each 600–3000 m^2 (about 10–50 m long), were selected to represent the availability of macrohabitat types in the study area. Two to four sections were sampled on summer and autumn sampling occasions. In winter, only one of the study sections was partially free of ice, and sampling in winter was thus more restricted than in other seasons. The winter section was, however, the main study section, which was sampled on all four occasions; at least 30% of observations came from this section on the summer sampling occasions also. Habitat availability among the study reaches did not differ appreciably (each included both fast-flowing riffles and slower flowing, deeper areas) at other sampling times, so we assume that the overall availability in April was adequately represented by the section sampled then. Although it is possible that some of our observations are not truly independent, we assume that, because of the time interval between sampling occasions, the probability of catching the same individual more than once was negligible (see also Heggenes et al. 1991).

After fish sampling was completed, six habitat variables were measured from each fish position: depth of water column, mean and surface water velocity, substrate composition (dominant and subdominant substrate size), and percent cover of instream vegetation. Depth of water was measured to the nearest centimeter using a wading rod. Mean and surface water velocities were measured at the 0.6 and 0.2 depths with a Schiltknecht Mini Water type 642 w-m/l flow meter fitted with a 20-mm propeller. Since we mainly used electrofishing for locating fish, we were not able to measure snout water velocities.

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Table 1. Habitat availability and use by different trout size-classes in the Raatekoski rapids, Kuusinkijoki River.

		Mean discharge ($m^3 \cdot s^{-1}$)	Water temperature (°C)	Depth (cm)	Dominant substrate (class)	Mean water velocity ($cm \cdot s^{-1}$)	Aquatic vegetation (% cover)	*N*
Midsummer	Availability	10.4	15–17	37 (1.2)	7 (5–8)	48 (1.8)	35 (2.1)	168
	4–9 cm			32 (2.2)	6 (5–8)	44 (4.0)	35 (5.3)	33
	10–15 cm			40 (3.7)	7 (5–8)	45 (5.2)	19 (4.4)	22
	16–25 cm			58 (2.3)	6 (5–8)	54 (4.9)	19 (3.6)	20
Late summer	Availability	16.9	11–15	35 (0.7)	7 (3–8)	50 (1.4)	44 (1.8)	256
	4–9 cm			34 (1.1)	7 (4–8)	50 (3.0)	56 (4.6)	43
	10–15 cm			40 (1.6)	7 (5–8)	47 (4.0)	51 (6.0)	27
	16–25 cm			49 (4.8)	7 (6–8)	36 (4.8)	16 (7.3)	12
Autumn	Availability	3.5	4–10	26 (1.2)	7 (5–8)	21 (1.8)	46 (2.2)	120
	4–9 cm			25 (1.4)	7 (5–8)	18 (3.2)	58 (5.6)	26
	10–15 cm			32 (2.1)	7 (5–8)	18 (2.2)	47 (6.1)	17
	16–25 cm			33 (3.0)	7 (6–8)	31 (5.2)	47 (8.0)	16
Winter	Availability	2.2	0–1	28 (0.8)	7 (5–8)	36 (2.0)	26 (2.1)	123
	4–9 cm			26 (2.2)	7 (5–8)	25 (3.0)	14 (3.7)	20
	10–15 cm			30 (2.0)	7 (6–8)	19 (2.7)	15 (2.9)	24
	16–25 cm			39 (3.0)	7 (7–8)	24 (4.6)	19 (3.1)	10

Note: The standard error is shown in parentheses following means of depth, mean water velocity and cover of aquatic vegetation, while the median value (with range given in parentheses) is given for the dominant substrate size. Substrate is classified according to the Wentworth scale (modified from Heggenes 1988): 1, 0.07–2 mm; 2, 2.1–8 mm; 3, 8.1–16 mm; 4, 16.1–32 mm; 5, 32.1–64 mm; 6, 64.1–128 mm; 7, 128.1–256 mm; 8, >256.1 mm.

However, we were mainly interested in documenting trout habitat preferences for future use in stream management programmes. As pointed out by Heggenes and Saltveit (1989), areas with suitable snout water velocities are rarely, if ever, limiting in trout streams; thus, a more general description of water velocities (e.g., mean water velocity) may be more informative for management purposes. Moreover, Baltz et al. (1987, 1991) have shown that in microhabitats used by rainbow trout (*Oncorhynchus mykiss*) the mean column velocity and focal point velocity are highly correlated. Substrate composition and percent cover of instream vegetation were determined visually in 0.8×0.8 m quadrats directly below the fish. We classified substrate particles according to a modified Wentworth scale, using categories 4–8 (sand–boulder) of the 13 particle-size categories in Heggenes' (1988) classification (4, 16.1–32 mm; 5, 32.1–64 mm; 6, 64.1–128 mm; 7, 128.1–256 mm; 8, >256.1 mm). Only dominant and subdominant substrate sizes were noted for each quadrat. Riparian vegetation was sparse and did not provide significant cover for trout. The only conspicuous form of instream cover available for trout was aquatic vegetation, which we quantified as percent cover per quadrat. To eliminate the potential bias introduced by interobserver variability, all measurements involving in-situ estimations were made by the same person (P. Kreivi).

Habitat availability was estimated using a stratified randomization protocol. In each study section, we positioned 5–15 equidistant transects perpendicular to the flow, and in randomly selected points along each transect we measured the same habitat characteristics as previously for fish positions. Habitat availability was assessed immediately after fish sampling; thus, each sampling occasion (measurement of habitat use and availability) was always completed in less than 1 week.

Analysis

Frequency-of-use histograms were constructed for each size-class × sampling time combination by calculating the relative frequency of use for increments of depth, mean water velocity, dominant substrate size, and percent cover of aquatic vegetation. Habitat preference was defined as (proportional use)/(proportional availability). Preferences were divided by the maximal preference value (i.e., habitat increment with the highest ratio of used to available habitat) so that the standardized suitability values would indicate a range of conditions from unsuitable (0.0) to optimal (1.0) ones (see Bovee 1982; Baltz 1990). A preference curve was then fitted to the data (i.e., preference values for each increment) by interpolation. For the preference analysis, the two summer sampling occasions, which gave qualitatively similar results, were pooled (see also Fig. 5, which shows that differences between the summer sampling occasions in habitat availability and use by trout were negligible); thus, only three seasons (summer, autumn, and winter) were considered here.

A two-way analysis of variance (ANOVA) was conducted for each of the four habitat variables (log-transformed data) to test for potential season × size-class interaction effects in trout habitat use. It is, however, highly unlikely that fish respond independently to different habitat characteristics. Instead, habitat selection is a complex, multivariate process whereby individuals evaluate their environment on the basis of the concurrent estimation of several, interacting variables (e.g., Shirvell and Dungey 1983; Heggenes and Saltveit 1989; Heggenes et al. 1991). Accordingly, we used multivariate approaches to detect seasonal and size-dependent changes in habitat preference of brown trout. Our methods were very similar to those used by Grossman and Freeman (1987) in a study of microhabitat selection by stream fishes. First, discriminant function analysis (DFA) was conducted to examine seasonal variation in (*i*) habitat availability and (*ii*) habitat use by trout. For simplicity, we combined trout size-classes for this analysis. ANOVA was performed on the canonical scores to find out whether seasonal differences in habitat availability or habitat use were statistically significant. In case of a significant overall difference, multiple comparisons using Tukey's test were made to identify significant pairwise differences. Principal components analysis (PCA) was employed to examine differences in habitat use between trout size-classes. Data from habitats actually used by trout and those from random sampling points were both included in the analysis to examine whether trout habitats constituted a nonrandom subset of the habitat available in the study reach. For this analysis, data were pooled over seasons. Size-class differences along the first two PCA axes were evaluated by ANOVA, followed by pairwise comparisons (Tukey's test). All data were appropriately transformed (log or arcsine) before entering the multivariate analysis.

Results

Habitat use and preference

Habitats used by brown trout were characterized by two

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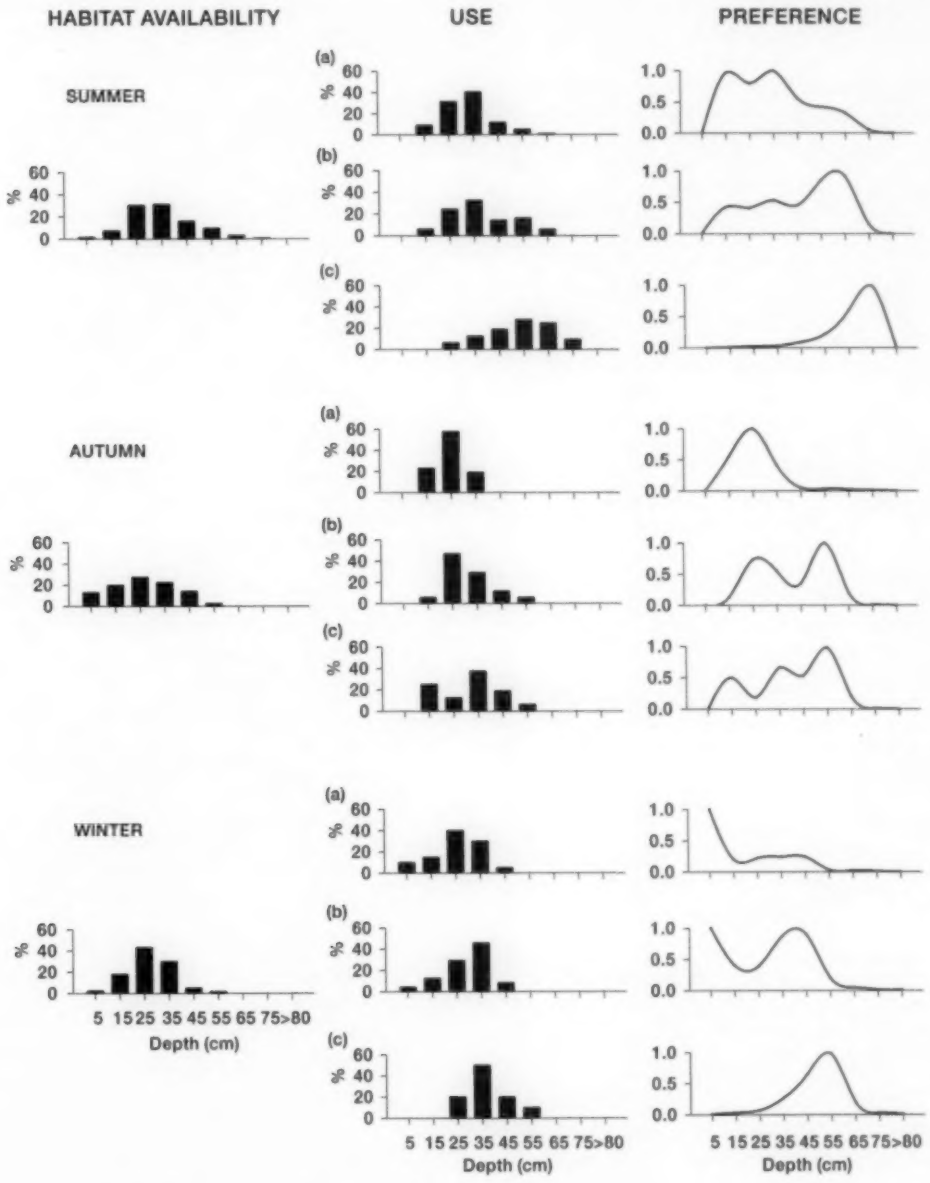
Fig. 1. Water depths available to, used by, and preferred by juvenile brown trout of (*a*) 4–9 cm, (*b*) 10–15 cm, and (*c*) 16–22 cm in three seasons.



consistent trends in relation to water depth: larger trout generally preferred deeper stream areas than smaller trout, and there was a shift toward shallower habitats in autumn by all trout size-classes (Fig. 1; note also the significant main effects of season and size-class in two-way ANOVA in Table 2). Depth availability, however, also became skewed toward shallow microhabitats in autumn, and even more so in winter, which at least partly explains the shift by trout to shallow stream areas at the onset of winter. Largest trout remained in the deepest available pools even during winter (see significant interaction term in Table 2). When observed over all seasons, the optimal ranges for depth were 5–35, 40–60, and 50–75 cm for small, medium, and large juvenile trout, respectively.

Trout used habitats with different water velocities in close relation to their availability. Moreover, there were only slight and nonsignificant differences in mean velocities used by different trout size-classes (Fig. 2, Table 2). In autumn trout larger than 16 cm preferred high-velocity habitats, whereas in winter all juvenile trout, especially the largest ones, clearly preferred slowly flowing stream areas. Seasonal differences between size-classes were, however, nonsignificant (see season × size interaction term in Table 2).

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In summer, trout used substrate categories almost as these were represented in the available habitat, with a slight preference for small substrates by all trout (Fig. 3). The largest trout exhibited an autumnal shift toward habitats with cobble or boulder substrates. In winter the two largest size-classes clearly preferred areas with the largest substrates available in the study reach. Even young-of-the-year trout showed a slight wintertime preference for coarser substrates (large pebbles to cobbles; Fig. 3). Trout size-classes did not differ in their substrate use (nonsignificant effect of fish size), and neither was there any indication of seasonal differences among size-classes, as indicated by the nonsignificant interaction term in two-way ANOVA (Table 2).

In summer and autumn the smallest fish favoured stream areas with a high percentage (80–100%) of aquatic vegetation. The largest trout, in contrast, occupied relatively vegetation-free habitats, especially during the summer (Fig. 4). In autumn the preference curves for instream vegetation were bimodal for larger trout (>10 cm): some specimens selected areas with very little aquatic vegetation, whereas others preferred habitats with a high (>60%) amount of vegetation. Finally, in winter, a distinct preference for low percentages of aquatic vegetation was evident in all trout size-classes. This, however, may partly reflect the spatial restrictions posed by sampling fish in difficult ice conditions in winter. Stream margins with dense growths of aquatic vegetation could not be effectively sampled in April, and habitat availability was thus primarily represented by central stream areas where the amount of vegetation was somewhat lower than in stream margins. Nonetheless, especially the largest trout showed complete avoidance of densely vegetated habitats in winter. Two-way ANOVA showed significant seasonal and size-class differences in the use of aquatic vegetation by trout. Notwithstanding the apparent seasonal differences among size-classes in their use of differently vegetated stream areas, the interaction term (season × size-class; see Table 2) was only marginally significant (p = 0.013; adjusted, table-wide 0.05 risk level after sequential Bonferroni correction, p = 0.01).

Table 2. Two-way ANOVAs for the effects of season and fish size-class on the habitat use of juvenile brown trout in relation to four environmental variables.

	Source of variation	F	df	p
Depth	Season	22.27	3	<0.001
	Size-class	37.20	2	<0.001
	Season × size-class	3.34	6	<0.05
	Error		257	
Mean water velocity	Season	30.42	3	<0.001
	Size-class	0.97	2	ns
	Season × size-class	2.15	6	ns
	Error		257	
Substrate size	Season	5.80	3	<0.01
	Size-class	3.19	2	ns
	Season × size-class	1.41	6	ns
	Error		257	
Aquatic vegetation	Season	20.14	3	<0.001
	Size-class	6.77	2	<0.01
	Season × size-class	2.77	6	ns
	Error		257	

Note: To control for type-I error rate when performing multiple tests, the sequential Bonferroni test (Holm 1979) was used to determine if results were significant at the table-wide level. ns, not significant (p > 0.05).

Seasonal variation in habitat availability and use

Discriminant analysis extracted two axes for seasonal variation in habitat availability (94.3% of variance explained) and use (92.8% of variance explained). The first axis for habitat availability was flow related, with high loadings for current velocity and depth (Fig. 5A). Habitat availability remained almost constant during summer, but velocities and water depths were generally lower in autumn and winter. DFA axis 2 had a heavy loading on the percent cover of vegetation. Winter was significantly different from other seasons along this axis because of low availability of aquatic vegetation.

DFA for resource use revealed some substantial seasonal changes in habitat use by brown trout. Axis one demonstrated an autumnal shift to shallow, low-velocity habitats with coarse substrates (Fig. 5B). However, except for the preference for boulder substrates, this trend mainly paralleled seasonal variation in stream habitat structure. Seasonal changes along DFA axis 2 were clearly related to instream cover of aquatic vegetation (Fig. 5B), but this also reflected a concurrent change in habitat availability. Habitats occupied by trout had a higher percentage of vegetation in late summer and in autumn than in other seasons, suggesting an important role for this variable in seasonal variation of trout habitat use.

Ontogenetic changes and nonrandom habitat use by trout size-classes

PCA on habitat availability and use (data pooled over seasons) extracted two components with eigenvalues >1.0 (Fig. 6). Component one loaded heavily on the current velocity variables and, to a lesser extent, on depth of water. Component two was mainly influenced by substrate characteristics, representing a gradient from vegetation-covered cobble substrates to coarser substrates with low amount of vegetation. ANOVA indicated size-related differences in habitat use only on component two, reflecting the frequent occupancy of stream areas with high amounts of vegetation by age-0 trout compared with larger fish (Fig. 6). There was some evidence of ontogenetic changes along the first component as well: larger trout seemed to prefer deeper, faster flowing habitats than smaller fish, but this trend was only marginally significant (one-way ANOVA for size-class differences on PCA axis 1, p = 0.086).

Trout of the smallest size category exhibited the clearest pattern of nonrandom habitat use. Microniches of these fishes, as represented by component one, were in slower water velocities and lower depths than those generally available in the stream habitat (Mann–Whitney U test on PCA scores, p = 0.021). Nonrandom distribution of subyearling trout was even more distinct along component two (Mann–Whitney U test, p = 0.003), indicating a preference for small substrates covered by submerged vegetation. Habitat use by larger trout differed significantly from availability only on component two (Mann–Whitney U test: medium size-class, p = 0.026; largest size-class, p < 0.001). These patterns resulted from the tendency of larger trout to occupy stream areas with cobble and boulder substrates and low amounts of instream vegetation.

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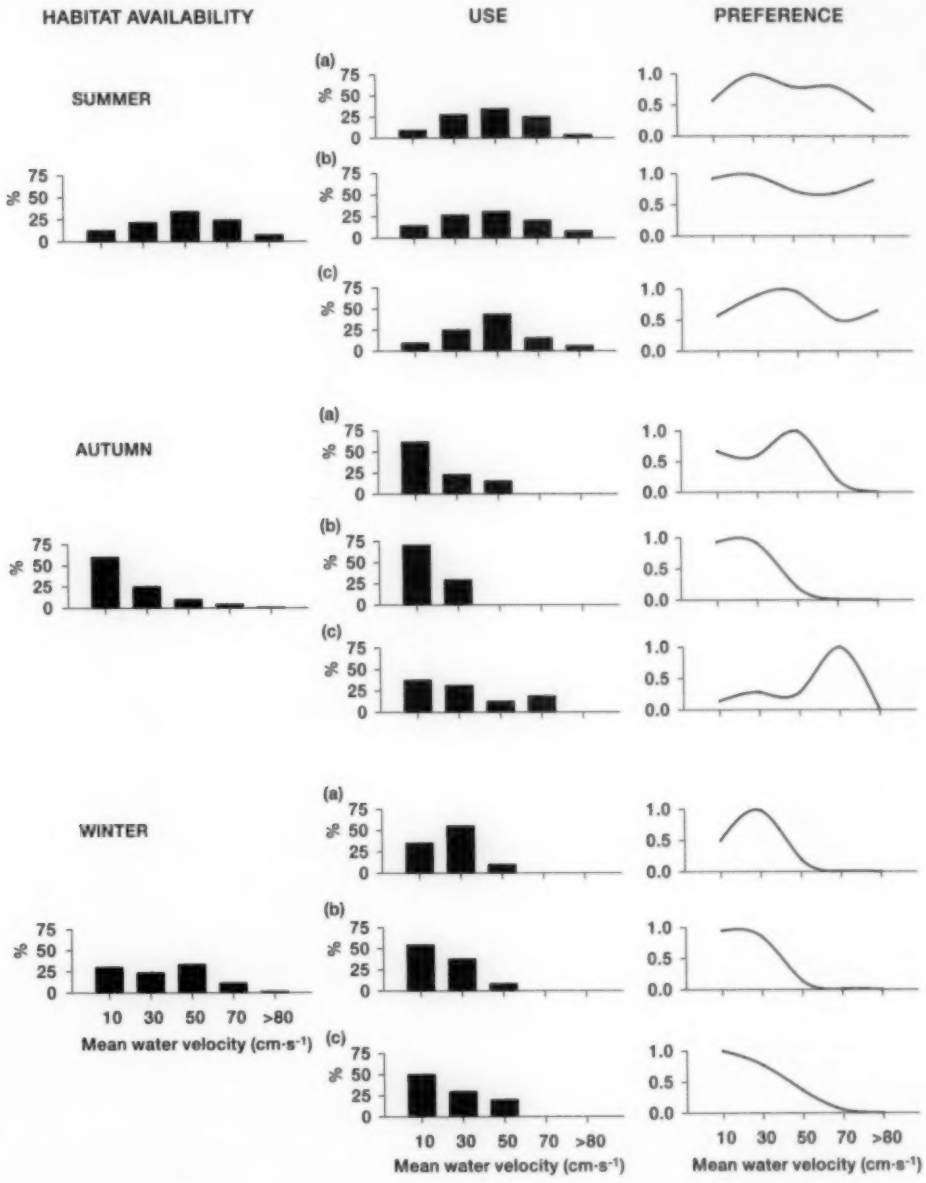
Fig. 2. Mean water velocities available to, used by, and preferred by juvenile brown trout of (*a*) 4–9 cm, (*b*) 10–15 cm, and (*c*) 16–22 cm in three seasons.



Discussion

The initial goal for developing habitat suitability functions for stream fishes was to establish universal curves, which could be readily transferred to diverse stream conditions (e.g., Bovee 1982). Recently, however, many authors have underlined the dynamic nature of fish habitat use, thus cautioning against generalizations based on single snapshot studies. It now seems evident that preference curves need to be developed in situ, and generalizations, if any, can be made only on a regional scale (Moyle and Baltz 1985; Heggenes and Saltveit 1989; Baltz et al. 1991; Greenberg et al. 1994). Salmonid fishes are quite plastic in their habitat use, responding flexibly to seasonal variations in habitat availability (Grossman and Freeman 1987; Heggenes and Saltveit 1989; Vondracek and Longanecker 1993). In addition to variation in availability, habitat use may be affected by a variety of other factors such as fish size (Bohlin 1977; Greenberg et al. 1994), time of day (Heggenes et al. 1993; Riehle and Griffith 1993; Shuler et al. 1994), mode of activity (Shirvell and Dungey 1983), discharge (Pert and Erman 1994), temperature (Baltz et al. 1987; Taylor 1988), food availability (Wilzbach 1985; Bridcut and Giller

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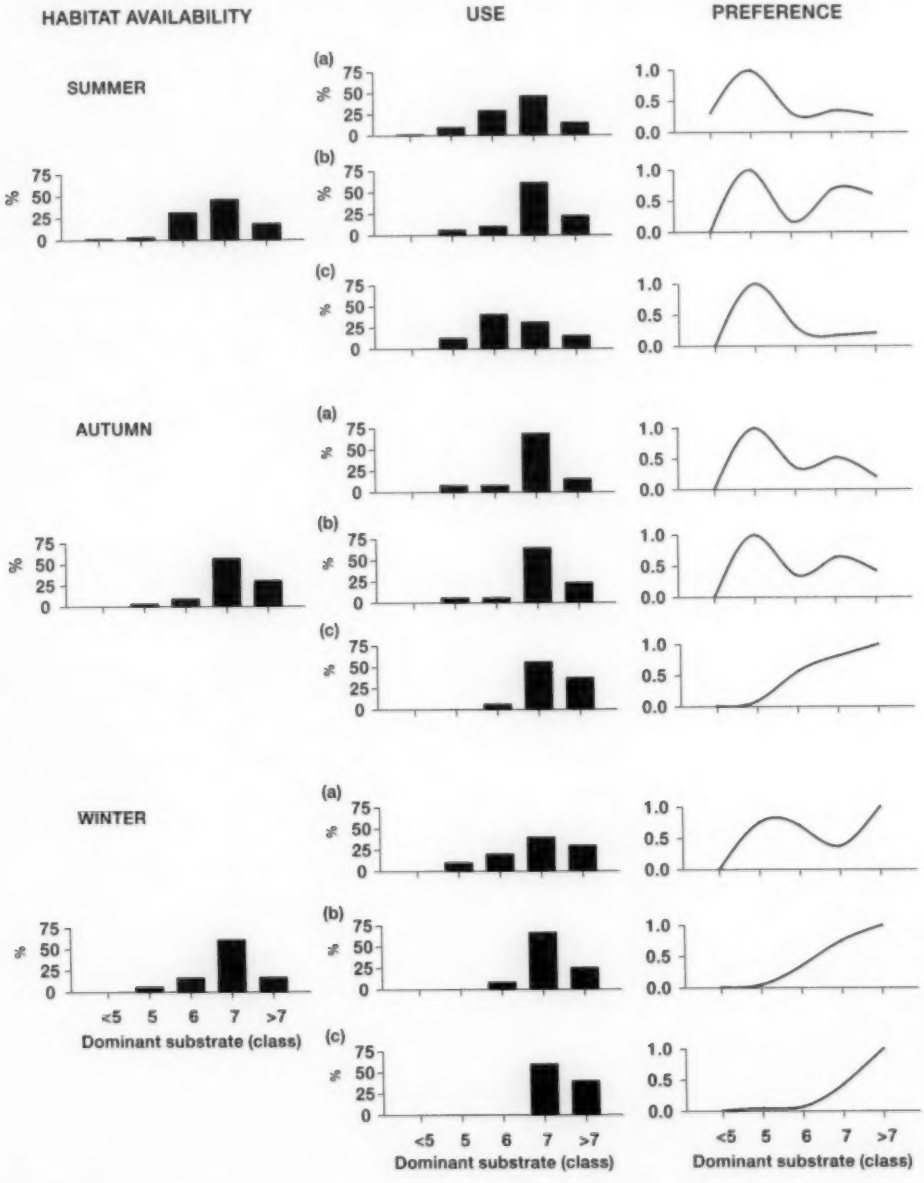
Fig. 3. Dominant substrate available to, used by, and preferred by juvenile brown trout of (*a*) 4–9 cm, (*b*) 10–15 cm, and (*c*) 16–22 cm in three seasons.



1993), presence of competitors (Fausch and White 1981; Grossman and Boule 1991), intraspecific interactions (Fausch 1984; Greenberg 1994), and risk of predation (Brown and Moyle 1991; Greenberg 1994).

Since so many factors can contribute to fish habitat use, it is hardly surprising that suitability curves based on summertime snapshot studies have met with limited success in habitat modelling applications (e.g., Heggenes 1994). There have been repeated demands to improve the predictive capacity of such models by developing suitability criteria stratified not only by fish size and season, but also by the feeding regime, time of day, habitat type, and discharge (Moyle and Baltz 1985; Orth 1987; Pert and Erman 1994; Vondracek and Longanecker 1993). Unfortunately, the construction of independent curves for all of the various aspects of fish habitat use will not be logistically feasible in most cases and could even result in considerable conflicts of interpretation. We suggest that the minimum requirement for increasing the biological realism of instream flow models is that the size structure of the fish population under study and the seasonality of resource availability and use be incorporated into the models. In streams of high northern areas the harsh winter conditions are far more

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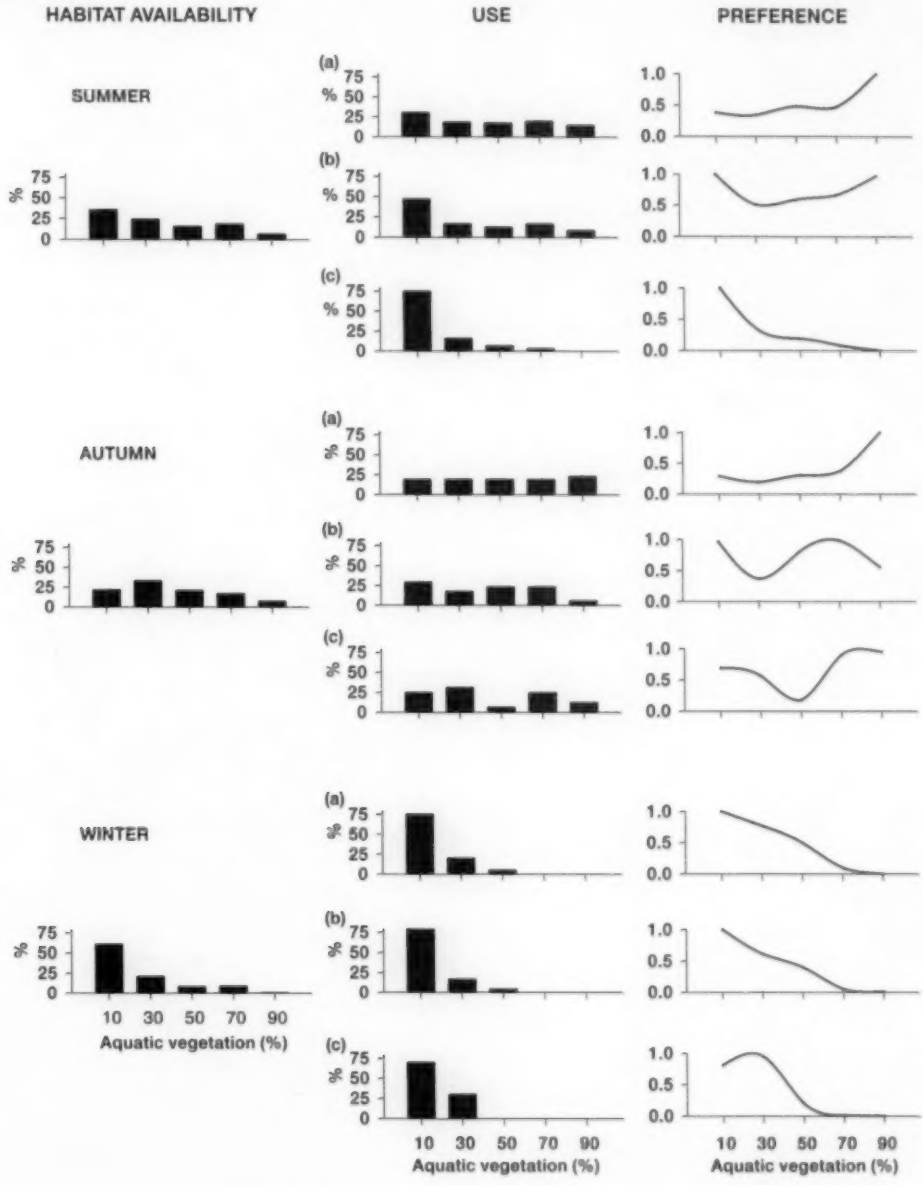
Fig. 4. Percent cover of aquatic vegetation available to, used by, and preferred by juvenile brown trout of (*a*) 4–9 cm, (*b*) 10–15 cm, and (*c*) 16–22 cm in three seasons.



likely to be critical for salmonid survival and production, and the development of separate suitability curves for winter is thus clearly advisable in such environments.

The instream flow incremental methodology (IFIM) is conventionally based on three life-history categories: fry, juvenile, and adult fish. Our results show, however, that these categories may fail to encompass the size-related variation in habitat use of salmonid fishes (see also Harris et al. 1992; Vondracek and Longanecker 1993). In the river Kuusinkijoki, adult trout larger than 25 cm were infrequent, and the trout population was dominated by smaller size-classes. Partitioning of the juvenile category into two size-classes (10–15, 16–22 cm) was clearly justified on the basis of size-related habitat shifts. The territorial system of brown trout is based on a social hierarchy whereby the largest individuals occupy the preferred low-velocity microhabitats, forcing smaller conspecifics to energetically less profitable stream positions (Bachmann 1984; Rincon and Lobon-Cervia 1993). It may be that, in the near absence of adult trout, the largest juvenile fish are able to increase their use of the pool habitats. Such a shift toward deeper habitats in the absence of large fish has also been observed in juvenile rainbow trout (Baltz et al. 1991).

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Fig. 5. Discriminant function analysis on seasonal changes in microhabitat availability (*a*) and use (*b*) by juvenile brown trout (size-classes pooled). Mean DFA scores with 95% confidence intervals are presented for midsummer (MS), late summer (LS), autumn (A), and winter (W) sampling occasions. Seasons connected with vertical or horizontal lines do not differ significantly (ANOVA followed by Tukey's pairwise comparisons). Variables with loadings >|0.35| are also given.

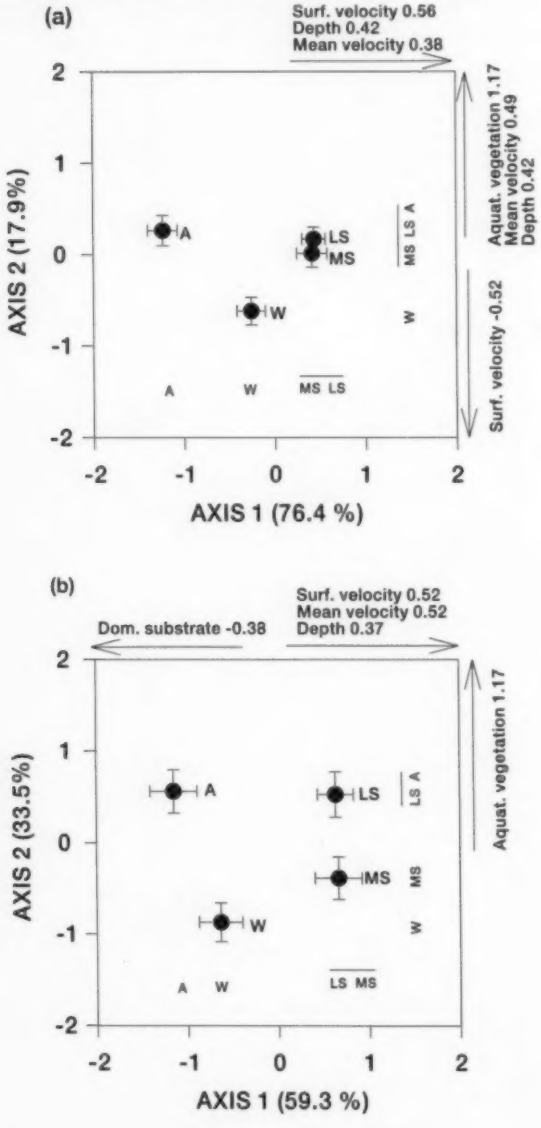
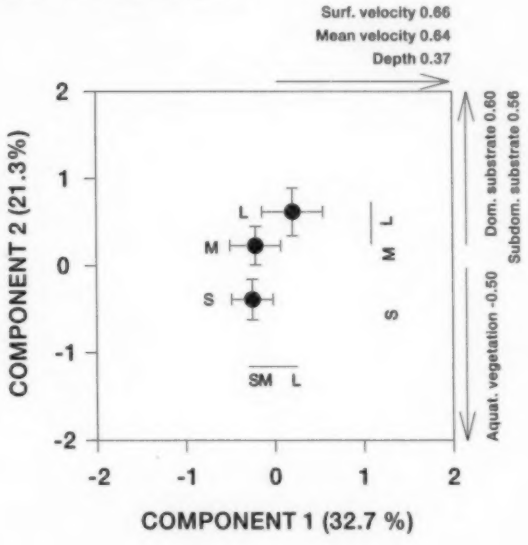


Fig. 6. PCA on size-class differences in habitat use by juvenile brown trout (seasons pooled). Mean PCA scores with 95% confidence intervals are presented for the size-classes 4–9 cm (S), 10–15 cm (M), and 16–22 cm (L). Size-classes connected with vertical or horizontal lines do not differ significantly (ANOVA followed by Tukey's pairwise comparisons). Variables with loadings >|0.35| are also given.



Many authors have suggested that the same mechanisms (competition, predation) underlying the fish size – habitat relationships in other stream fishes are also operating in salmonids (Bohlin 1977; Heggenes 1989; Greenberg et al. 1994). Accordingly, juvenile trout might simply be making the best of a bad situation; since they have no access to preferred pool habitats, they choose to dwell in low-velocity microhabitats along stream margins, yet in the vicinity of profitable feeding areas further away from the stream edge (e.g., Fausch 1984; Shuler et al. 1994). Recent experiments by Greenberg (1994) showed, however, that juvenile brown trout (ca. 12-cm average size) preferred fast-flowing riffles and runs to deeper, slow-flowing run margins and pools, which were used only when trout densities were high. The presence of northern pike (*Esox lucius*), a fish predator, further decreased the use of pools where the pike mainly occurred. Thus, it may be that juvenile trout are not hierarchically excluded from stream pools by bigger fish; instead, predator-free riffle margins offering both suitable refuge sites and nearby feeding areas may represent the best combination of habitat conditions for juveniles in many trout streams. The few adult trout and grayling that occurred in our study area probably induced a localized predation threat, forcing the youngest individuals away from the stream pools. The largest juveniles, in contrast, may have been able to expand their use of stream habitat because of a size refuge from predation. Unfortunately, virtually nothing is presently known about the effects of size-dependent predation risk on the habitat use of salmonid fishes.

Many previous studies of habitat use by salmonid fishes have used sampling stratified by fish size or season. Few, if any, however, have included both of these aspects, thus complicating any comparisons with previous studies. In our study, multivariate analysis ranked water velocity as the most important variable in differentiating among habitats used by trout at different seasons and also among habitats used by different trout size-classes. Water depth also contributed to seasonal and size-class differences but much less importantly than velocity. Similar rankings of physical variables in trout microhabitat use

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have been reported by Gatz et al. (1987) and Heggenes and Saltveit (1989).

Summertime habitat use and preference by differently sized trout was generally well in accordance with previous findings (for reviews, see Heggenes 1989, 1994). Thus, the frequently documented bigger fish – deeper habitat pattern was distinct in all seasons: the largest fish always preferred the deepest water available, whereas young-of-the-year trout were mainly found in relatively shallow riffle areas. Habitats with different water column velocities were used almost at the frequency at which they were available in the stream habitat, whereas all size-classes showed preference for relatively small substrate particles (large gravel to small cobble). The largest trout seemed to avoid habitats with high percentages of instream vegetation, but this may have been a side effect of an active search for deep, slow-flowing pool habitats. Young-of-the-year trout, by contrast, intensively used areas with a high percentage of instream vegetation in summer.

A distinct shift in habitat use by all size-classes occurred in winter. The first indications of the change could be seen in late fall when water temperature dropped below 10°C, but the habitat shift was not completed until winter. The spatial niche of all trout size-classes was considerably narrower in winter than in summer, as was also noted by Heggenes (1994). Young-of-the-year trout selected shallow (mainly less than 25 cm) stream areas with much lower mean water velocities than in their summer habitats. The winter habitats of larger trout were in relatively deep water, but they also chose positions in low-velocity habitats with a coarse substratum and little underwater vegetation. According to Heggenes (1994), trout abandon shallow habitats in winter to avoid freezing and dewatering. In contrast, Bustard and Narver (1975) mainly found fry of steelhead trout (*Oncorhynchus mykiss*) in shallow water (<15 cm) near stream margins during winter, and cutthroat trout (*Oncorhynchus clarki*) also inhabit shallower stream areas in winter than in summer (Heggenes et al. 1991). We share the view of Cunjak and Power (1987) that depth is probably not a primary factor in winter habitat selection of trout. Instead, they prefer areas that provide reduced flows or ample cover in the form of submerged vegetation or interstitial crevices among coarse substrates (see also Heggenes et al. 1993). In the present study, however, no wintertime preference for instream vegetation was observed, but, as expected, there was a shift toward microhabitats with coarser substrates during winter. Avoidance of macrophyte beds by all juvenile trout was somewhat surprising, because many previous studies have documented intensive use of instream cover by juvenile salmonids in winter (Bustard and Narver 1975; Rimmer et al. 1983, 1984; Cunjak and Power 1987; Taylor 1988; Riehle and Griffith 1993). However, wintering trout are also known to shelter within the interstitial spaces of coarse substrate particles (Bjornn 1971; Hillman et al. 1987), which has led some authors to suggest that the availability of such particles is the prime determinant of the suitability of a stream area as a wintering grounds for salmonid fishes (Rimmer et al. 1984; Heggenes et al. 1993). In winter, trout adopt a shelter-and-move strategy whereby they avoid the risks of ice damage by being active at night and sheltering within the substrate during the day (Heggenes et al. 1993). Since all trout size-classes clearly preferred the coarsest substrates available in our study reach, this creates potential for intensive intraspecific competition among brown trout during winter (see also Heggenes et al. 1993). It is therefore essential that stream management programmes in areas with severe winter conditions ensure that appropriately sized (cobble–boulder) substrates are abundantly available in trout wintering areas.

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Settlement timing, distribution, and abundance of Dover sole (*Microstomus pacificus*) on an outer continental shelf nursery area

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Abstract: Settlement timing, distribution, and abundance of Dover sole (*Microstomus pacificus*) were compared with depth and environmental variables off Oregon, U.S.A. Surveys were made every 2 months from January to November 1989 on three 50–400 m depth transects and every March from 1990 to 1994 on one transect. Annual March age-1 catch per unit effort (CPUE) estimates did not support Hayman and Tyler's (R.A Hayman and A. Tyler. 1980. Trans. Am. Fish. Soc. 109: 54–70) hypothesis that strong year-classes are associated with offshore convergence during the settlement season, despite a similar distribution of oceanographic conditions during the two studies. High March CPUE was correlated with high bottom temperature in the primary nursery area (100–119 m depth) and an increase in the proportion of larvae in deeper strata. Seasonal and annual observations support Hayman and Tyler's assumption that the primary postsettlement nursery area is bathymetrically restricted. Otolith increments indicated that larvae at 50–159 m had been on the bottom longer than larvae from greater depths, suggesting postsettlement inshore migration. Sediment size and bottom temperature do not explain the high CPUE in the primary nursery area. A negative size–depth relationship and a change in depth distribution with increasing density suggest that biological interactions may influence the spring distribution.

Résumé : Le moment de l'établissement sur le fond, la distribution et l'abondance de la limande-sole (*Microstomus pacificus*) ont été comparés à des variables bathymétriques et environnementales au large des côtes de l'Oregon (États-Unis). Des relevés ont été effectués tous les 2 mois de janvier à novembre 1989 sur trois transects d'une profondeur de 50–400 m, et tous les mois de mars de 1990 à 1994 sur un seul transect. Les estimations annuelles des indices des prises par unité d'effort de poissons d'âge 1 en mars n'ont pas confirmé l'hypothèse de Hayman et Tyler (R.A Hayman et A. Tyler. 1980. Trans. Am. Fish. Soc. 109: 54–70) selon laquelle les fortes classes seraient associées à une convergence dans les eaux du large pendant la saison d'établissement sur le fond, malgré une distribution similaire des conditions océanographiques au cours des deux études. Le fort indice de prise par unité d'effort de mars était corrélé à une température au fond élevée dans la principale zone d'alevinage (profondeur de 100–119 m) et à une augmentation de la proportion de larves dans les strates plus profondes. Les observations saisonnières et annuelles confirment l'hypothèse de Hayman et Tyler selon laquelle la principale zone d'alevinage après établissement sur le fond est restreinte sur le plan bathymétrique. Les anneaux de croissance des otolithes indiquent que les larves se trouvant dans la strate 50–159 m étaient restées sur le fond plus longtemps que les larves provenant des plus grandes profondeurs, ce qui semble indiquer une migration vers les côtes après l'établissement sur le fond. La granulométrie des sédiments et la température du fond n'expliquent pas le niveau élevé des prises par unité d'effort dans la principale zone d'alevinage. La relation négative entre la taille et la profondeur d'une part, et le changement observé dans la distribution en profondeur en fonction de l'accroissement de la densité d'autre part, permettent de penser que des interactions biologiques peuvent avoir un effet sur la distribution printanière.
[Traduit par la Rédaction]

Introduction

Dover sole (*Microstomus pacificus*) is a commercially important flatfish, which ranges from the Bering Sea to Baja California at depths of 10–1189 m (Allen and Smith 1988). Larvae are planktonic for at least a year before settling on the outer continental shelf during the winter and early spring (Pearcy et al. 1977; Markle et al. 1992; Toole et al. 1993; Butler et al. 1996). Larvae complete metamorphosis approximately 3–6 months after settlement (Markle et al. 1992; Toole et al. 1993; Butler et al. 1996). Most Dover sole reach reproductive maturity and enter the fishery approximately 6–7 years after settlement (Hunter et al. 1992; Turnock et al. 1994).

General distributional patterns of adult Dover sole are well known. A positive relationship between average size and depth and a seasonal inshore (summer) – offshore (winter) movement have been described (Hunter et al. 1990; Westrheim et al. 1992; Jacobson and Hunter 1993). However, descriptions of juvenile distribution and abundance are limited (Demory 1971; Pearcy 1978; Markle et al. 1992). This is especially true for the most recently settled age-class. For example, Pearcy et al. (1977) described the distribution of new recruits as the "outer shelf." Markle et al. (1992) provided information regarding the depth of new recruits during January and March

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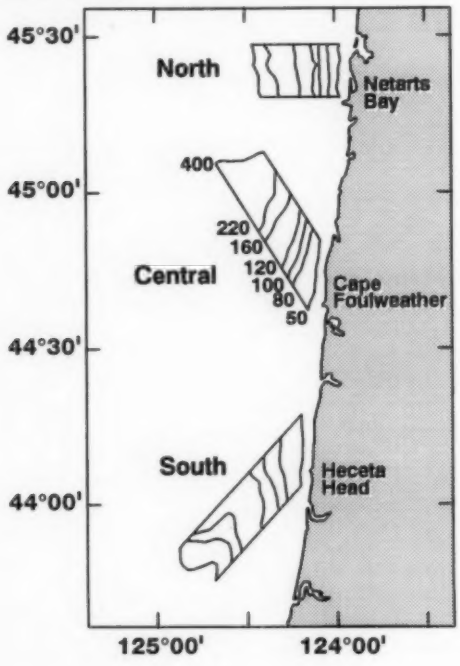
Fig. 1. Location of bottom trawl transects off the Oregon coast. Contours are boundaries of depth strata (m).



1989 but did not relate specimen numbers to catch per unit effort (CPUE) or address seasonal or annual variation.

Settlement timing and distribution of new recruits are important because the early juvenile phase of marine fishes may be critical in determining year-class strength (e.g., Sissenwine 1984; Peterman et al. 1988; Bradford 1992). Blaxter (1988) proposed that metamorphosis at the end of the larval period may represent a critical period, during which mortality caused by predation may be particularly high. High mortality may occur for species that change habitats by settling from the pelagic zone to the bottom during metamorphosis if the fish are not successful in locating appropriate prey and refuges from predation (Haldorson and Richards 1987; Hixon 1991; Walters and Juanes 1993).

Hayman and Tyler (1980) proposed a mechanism that relates Dover sole year-class strength to the settlement process. They described a significant correlation between abundance of age-6 female Dover sole and offshore convergence (stronger downwelling offshore than inshore) in January of the settlement year. Hayman and Tyler (1980) hypothesized that offshore convergence restricts settling larvae to the outer continental shelf, and they suggested that there is a selective advantage to settling in this area. They proposed that the advantage may be related to food or physiological requirements during an initial postsettlement growth period.

Our study describes the distribution of recent recruits and older juvenile Dover sole in relation to depth and physical environmental variables. This information is a prerequisite for further evaluation of Hayman and Tyler's (1980) recruitment hypothesis. We describe seasonal settlement timing, distribution, and abundance during a single year, 1989, and annual variation based upon March samples for the 6-year period 1989–1994.

Methods

Terminology

We refer to the most recently settled age-class of Dover sole as age 1 because larvae are planktonic for a year or more (Pearcy et al. 1977; Markle et al. 1992; Toole et al. 1993), placing their birth at least 1 calendar year before collection. Some larvae may be older than 1 year at the time of settlement (Markle et al. 1992), and Butler et al. (1996) believe that settlement may take place between 9 and 29 months after hatching. Classifying new recruits as age 1 matches the convention used when ageing adult Dover sole (Chilton and Beamish 1982).

Length measurements were standard length (SL). Conversion to total length (TL) over the range of sizes collected during this study is described by TL (mm) = 0.26 + 1.191(SL (mm)) (r^2 = 0.996; P < 0.001).

Survey design and estimation of abundance

Dover sole were collected in bottom-trawl surveys conducted during alternating months, January–November 1989, on three transects along the Oregon coast (Fig. 1; Markle et al. 1992). Additionally, one March survey was conducted along the central transect each year between 1990 and 1994. Each transect was 18.5 km wide and oriented approximately perpendicular to shelf isobaths. Transects ranged from 50 to 400 m and were divided into six depth strata bounded by isobaths at 50, 80, 100, 120, 160, 220, and 400 m. Untrawlable rocky areas were not sampled.

A minimum of three 5-min tows was attempted in each depth stratum of each transect. Tow locations (stations) were selected at random and were made parallel to bathymetric contours. The actual number of successful tows varied between 0 and 12 per stratum (Appendix). More tows were attempted between 80 and 159 m, where Dover sole abundance and (or) variability were greatest, and fewer tows were successfully completed in the 220- to 400-m stratum, which required the most ship time to sample.

All trawling was conducted during daylight hours using a commercial, 34.9-mm mesh, two-seam shrimp trawl with a 27.4-m headrope and a 28.5-m footrope and tickler chain. The posterior three quarters of the cod end had a 6.4-mm liner. Area sampled during individual tows could not be determined accurately, so catch is reported as number per 5-min tow (CPUE). The sampling unit (Cochran 1977) necessary for calculating the variance of combined-stratum means was estimated to be 0.005 km^2/tow, on the basis of mean tow distance and an assumption that the net opened to 75% of the headrope length.

Size selectivity of the fishing gear was evaluated indirectly. It is likely that the smallest benthic Dover sole were retained because the 11-mm minimum body depth (Markle et al. 1992) greatly exceeded the cod-end mesh size. However, large fish could avoid the fishing gear, on the basis of comparison with Dover sole length frequencies from 9-cm mesh groundfish bottom trawls fished in the study area during the summer of 1989 (Weinberg et al. 1994; Toole 1994). To reduce the bias of low selectivity for larger fish, only Dover sole that were ≤210 mm were considered in this study.

The distribution of CPUE was significantly non-normal (Komolgorov–Smirnov goodness of fit; P = 0.001) and not significantly different from a lognormal distribution (P = 0.221) when strata containing the highest spring age-1 catches (100–159 m) were aggregated. Station catches were transformed by ln(CPUE + 1) in an attempt to normalize data for statistical tests. Transformed station catches were entered directly into an analysis of variance (ANOVA) for 1989 observations and the model calculated factor means that, when back-transformed to original units, corresponded to geometric means. For among-year comparisons, stratum means were estimated

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from transformed station catches using the Finney–Sichel maximum-likelihood estimator (MLE) for lognormal data (McConnaughey and Conquest 1993). This unbiased estimator is slightly higher than the geometric mean. Among-year transect (combined strata) means and associated variances were derived from stratum MLEs, weighted by the number of 0.005-km^2 sampling units within each depth stratum, the total sampling units within all strata, and the number of units sampled (Cochran 1977; Pennington and Brown 1981).

Age and ontogenetic stage determination

The catch was sorted on board and nearly all Dover sole ≤210 mm were counted, frozen or fixed in formalin, and returned to the laboratory. In the laboratory, Dover sole were measured to the nearest millimetre and staged according to criteria of Markle et al. (1992). Briefly, stage 3 includes the most recently settled larvae, stage 4 includes late-metamorphic demersal larvae, and stage 5 includes fully transformed juveniles.

Otoliths were removed from subsamples of Dover sole collected during 1989 and 1990 and from all frozen Dover sole in 1991–1994. Whole otoliths were examined under reflected light against a black background and translucent postsettlement annuli (Toole et al. 1993) counted. For each survey, an age–length key was developed to estimate the age of all Dover sole from the sample of aged fish. The method was modified to take advantage of the staging procedure (Toole 1994), because all frozen fish were staged and all stage-3 and stage-4 larvae were age 1.

Settlement timing

Distributions of settling dates were determined for 1989–1993 surveys, on the basis of subsamples of otoliths from March age-1 Dover sole. An index of settling date was estimated for each fish by counting daily increments distal to the point at which growth from accessory primordia first encloses growth from the central primordium (Toole et al. 1993). This structural landmark is closely associated with the beginning of stage 3, when Dover sole first begin to spend a significant amount of time on the bottom. Toole et al. (1993) validated periodicity of postsettlement growth increments.

The proportion of stage-3 larvae in a sample also was evaluated as an index of settlement timing. While presumably less precise than estimates based on otolith increments, it is easier to determine and was available for all collections. Annual transect means were calculated as weighted averages of stratum means because settlement timing varied with depth (see Results).

Environmental factors

Bottom temperature was obtained for each tow during the March 1991–1994 surveys and for a few tows during the March 1990 survey. An index of mean monthly bottom temperature was available at two sites (55 and 100 m) within the central transect for the entire study period. This index was derived from mean monthly sea level height at Neah Bay, Washington (National Ocean Survey, Rockville, Md.), by applying equations in Table 2 of Kruse and Huyer (1983).

Monthly means of Bakun's (1973) index of the offshore component of Ekman transport (upwelling) and index of vertical velocity were obtained for a region centered at 45°N and 125°W (National Marine Fisheries Service (NMFS) Pacific Fisheries Environmental Group, Monterey, Calif.). This region encompasses all three transects. The index of vertical velocity represents the speed at which deeper water must rise to replace surface water that has been displaced by divergence, without accounting for coastal interference. Hayman and Tyler's (1980) convergence relationship was based on January vertical velocity at 46°N and 125°W.

Mean sediment grain size was estimated for each stratum from 179 sediment samples collected within the study area by Runge (1966). Original units are median ϕ, which is $-\log_2$ median diameter in millimetres (Inman 1952). Between 6 and 28 sediment samples were available for each depth stratum in the central and southern transects; however, only 1–7 samples were available for northern transect depth strata.

Table 1. Summary of three-way ANOVA results for Dover sole (*Microstomus pacificus*) during 1989 surveys ($N = 312$).

Source of variation	df	*P*
Age-1 Dover sole		
Main effects		
Month	5	<0.001
Transect	2	<0.001
Depth	5	<0.001
Interactions		
Month × transect	10	0.011
Month × depth	25	0.002
Transect × depth	10	0.635
Residual	254	
Total	311	
Age-2 to -5 Dover sole		
Main effects		
Month	5	0.003
Transect	2	0.522
Depth	5	<0.001
Interactions		
Month × transect	10	0.103
Month × depth	25	<0.001
Transect × depth	10	0.011
Residual	254	
Total	311	

Note: Dependent variable in all cases was ln(number/tow + 1).

Statistical tests

Comparisons were made among means of categorical variables with analysis of variance. Generally, depth was treated as a categorical variable because the response was distinctly nonlinear. If significance was detected, actual differences were shown with the Student–Newman–Keuls (SNK) multiple range test (e.g., Sokal and Rohlf 1969). An approximate test of the equality of means (Sokal and Rohlf 1969, p. 372) was performed when the assumption of homogeneity of variance could not be met (Bartlett's test). This test weights means according to the reciprocal of the variance of the sample from which they are taken and includes an error mean squares term that takes the weighting into account.

Continuous variables were analyzed with linear regressions. The effect of collection year on the relationship between temperature and depth was evaluated with a partial *F* test for a multiple regression containing dummy variables corresponding to years (Neter et al. 1989).

Results

Seasonal distribution and abundance during 1989

Age-1 Dover sole

CPUE of age-1 Dover sole in 1989 differed significantly among the 6 months, three transects, and six depth strata (Table 1). Interactions between month and transect and between month and depth also were significant; however, interactions between transect and depth were not.

March CPUE was significantly higher than the catch rate in other months (Fig. 2). March coincided with the peak

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Fig. 2. Monthly mean CPUE and 95% confidence intervals for age-1 and age-2 to -5 (≤210 mm) Dover sole (*Microstomus pacificus*); pooled by depth stratum and transect. Numbers of tows are indicated.

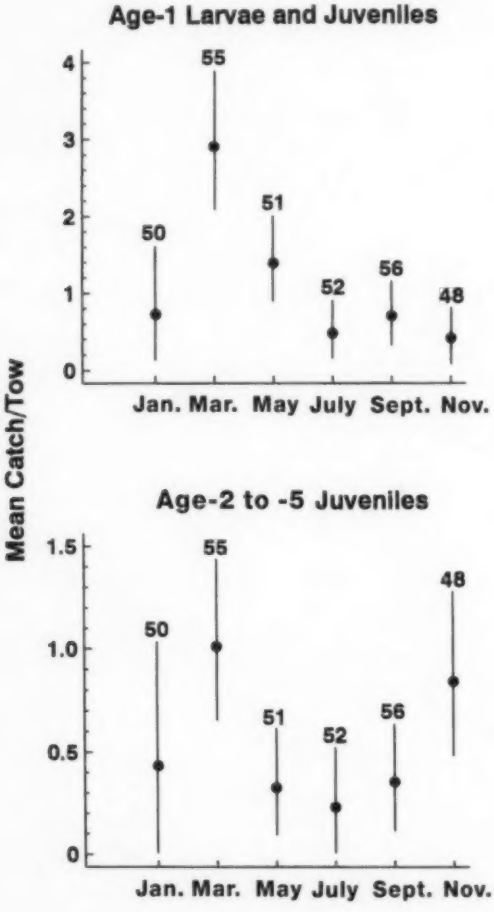
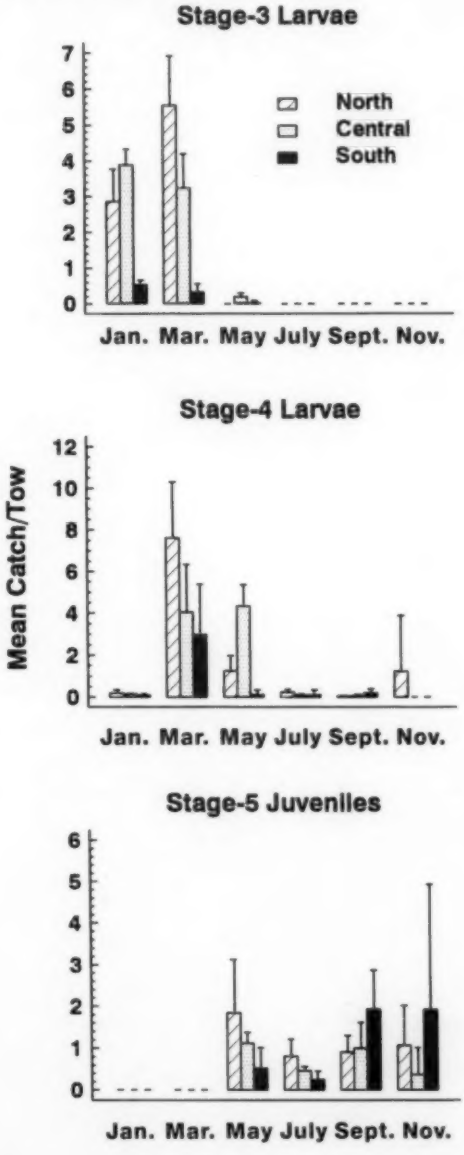


Fig. 3. Mean CPUE of age-1 Dover sole (*Microstomus pacificus*) by metamorphic stage, month, and transect in 1989. Error bars show 95% confidence intervals.



settlement period of larval Dover sole. Recently settled stage-3 larvae were most abundant in January and March, stage-4 larvae were most abundant in March and May, and stage-5 juveniles were caught between May and November (Fig. 3).

Age-1 CPUE was lower on the southern transect than on the others (Fig. 4). Initial settlement of larvae during the spring was lower on this transect than on others, on the basis of the significant interaction between month and transect and inspection of Fig. 3. However, by the fall, CPUE of stage-5 juveniles on the southern transect was equal to, or greater than, CPUE on other transects (Fig. 3).

Differences in CPUE among depth strata reflect a peak catch of age-1 Dover sole in the 100–119 m depth stratum (Fig. 5). When all transects and months were combined, three homogeneous groups of depth strata (in order of decreasing CPUE) were identified in multiple range tests: (*i*) 100–119 m; (*ii*) 80–99 and 120–159 m; and (*iii*) 50–79, 160–219, and 220–400 m. This pattern characterizes age-1 depth distribution during the larval settlement period, when CPUE was highest. However, age-1 Dover sole moved inshore during the summer and fall and returned offshore by late fall or winter. For example, on the central transect in 1989, CPUE of age-1 Dover sole was highest between 100 and 159 m from January through May, between 80 and 119 m in July, and inshore of 80 m in September (Fig. 6). By November, age-1 Dover sole again were caught at depths between 100 and 119 m.

Age-2 to -5 Dover sole

Differences in CPUE among months and depth strata were also significant for Dover sole ≤210 mm that had been on the

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Fig. 4. Transect mean CPUE and 95% confidence intervals of age-1 and age-2 to -5 (≤210 mm) Dover sole (*Microstomus pacificus*), pooled by month and depth stratum. Numbers of tows are indicated.

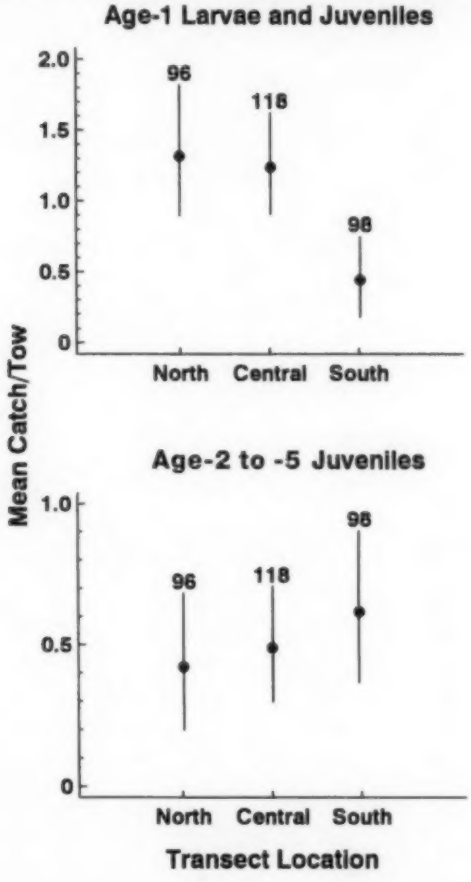
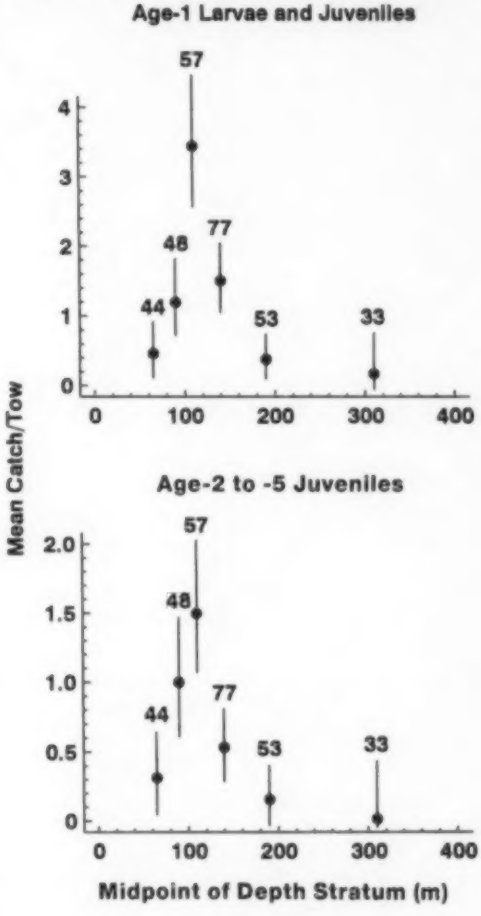


Fig. 5. Mean CPUE of age-1 and age-2 to -5 (≤210 mm) Dover sole (*Microstomus pacificus*) according to depth stratum, pooled by month and transect. Numbers of tows are indicated.



bottom 1 year or more (Table 1). However, significant differences among transects could not be detected. Interactions between month and depth and between transect and depth were significant, while interactions between month and transect were not significant.

CPUE of age-2 and older Dover sole within the sampling area was highest in March and November and lowest between May and September (Fig. 2). The low mean catch rate in January 1989 may reflect our inability to sample depths between 50 and 100 m on the northern transect during that survey (Appendix).

Mean CPUE for combined months was higher in the 80–119 m depth stratum than in the 50–79 or 160–400 m depth strata (Fig. 5). This depth distribution characterizes the winter and early spring period, when age-2 to -5 juveniles were found farthest offshore and well within the depth range surveyed. Like age-1 Dover sole, age-2 to -5 juveniles moved inshore during the summer (Fig. 6). The September distribution suggests that juvenile Dover sole may have moved to depths that were not sampled.

Age-2 to -5 Dover sole moved inshore earlier than age-1 Dover sole. The initial change in depth distribution and decline in age-2 to -5 CPUE within the study area occurred between March and May while that of age-1 Dover sole occurred between May and July (Fig. 6).

The highest CPUE of age-2 and older cohorts occurred at shallower depths than the highest CPUE of age-1 Dover sole during five of the six 1989 central transect surveys (Fig. 6). A negative relationship between standard length and average stratum depth was detected in all months except July and November ($r = -0.18$ to -0.58; $P < 0.001$ to 0.05).

1989 distribution and abundance in relation to environmental factors

Seasonal changes in abundance and distribution of larval and juvenile Dover sole corresponded with seasonal environmental patterns (Fig. 7). Onshore Ekman transport (downwelling), positive vertical velocity (offshore divergence), and warm bottom temperatures were predominant from January through March, when larvae were settling and peak CPUE was near the 100-m isobath. A transition occurred in April. Offshore Ekman transport (upwelling), negative vertical velocity

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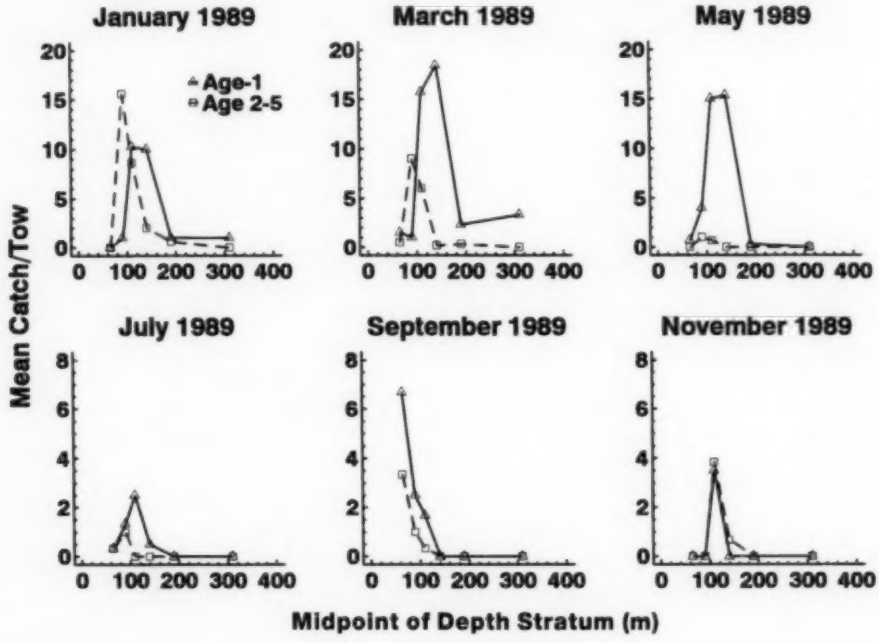
Fig. 6. Mean CPUE of age-1 and age-2 to -5 (≤210 mm) Dover sole (*Microstomus pacificus*) by depth stratum within the central transect in 1989.



(offshore convergence), and cooler bottom temperatures were predominant between May and September, when Dover sole moved into shallower water. The winter pattern again occurred from October through December, when juvenile Dover sole returned offshore.

Sediment size ranged from coarse sand (median ϕ diameter <2) in the shallowest depth strata to mud (median ϕ diameter >4) in deeper areas. Significant differences among the north, central, and south transects could not be detected. Differences in mean median ϕ diameter among depth strata were significant (ANOVA, $P < 0.001$). Multiple range tests identified three homogeneous groups of depth strata (in order of decreasing sediment size): 50–99, 100–159, and 120–400 m. The mean median ϕ diameters associated with each homogeneous group were 2.4, 3.4, and 4.0, respectively.

Annual variation in settlement distribution, abundance, and timing, March 1989–1994

Distribution and abundance

Central transect age-1 mean CPUE differed annually among 1989–1994 March surveys (equality of means test, $P = 0.006$; Fig. 8). Multiple range tests identified significantly higher CPUE in 1990 than in 1991, 1992, or 1994 and significantly higher CPUE in 1989 than in 1991. Correlation between mean transect density and mean January vertical velocity was not significant ($r = -0.31$; $P = 0.55$), despite conditions similar to those considered by Hayman and Tyler (1980) (Komolgorov–Smirnov goodness of fit; $P = 0.81$; Fig. 9). Correlations also were not significant during the settling months of November, December, and February.

Depth stratum and year interactions were not significant; however, years of higher transect CPUE generally corresponded to years in which deep stratum CPUE increased (Fig. 10). Stratum mean CPUE and transect mean CPUE were positively correlated for the three depth strata between 120 and 400 m ($r = 0.92$–0.96; $P = 0.002$–0.008) but were uncorrelated for the three strata between 50 and 119 m ($P = 0.13$–0.57). Strong offshore divergence in February and a deeper distribution (indexed by the ratio of mean CPUE in the 120- to 159-m stratum to the 100- to 119-m stratum) were correlated ($r = 0.88$; $P = 0.025$). Significant correlations were not observed in November–January.

Temperature at depth varied significantly among the four surveys (1991–1994) for which tow-specific temperatures were available (partial F test, $P = 0.003$; Fig. 11). Temperatures were generally higher in 1992 and 1993 than in 1991. A broad range of temperatures was observed at depths <120 m in 1994, possibly because conditions changed after the first leg of the survey. Age-1 Dover sole were collected at temperatures between 5.6 and 10.6°C, with the highest 20% of positive tows (>15 fish/tow) occurring between 7.4 and 10.3°C. Age-2 to -5 Dover sole were collected at temperatures between 7.5 and 10.7°C, with the highest 20% of positive tows (>18 fish/tow) occurring between 7.9 and 10.6°C. CPUE in the main nursery area (100–119 m for age 1 and 80–119 m for age 2–5) was positively correlated with bottom temperature (age 1: $r = 0.46$, $P < 0.001$; age 2–5: $r = 0.42$, $P = 0.001$).

Distribution and timing

The mean number of days since settlement varied among depth strata (ANOVA, $P < 0.001$), on the basis of a subsample of otoliths from combined March 1989–1993 surveys (Fig. 12). Multiple range tests indicated that age-1 Dover sole collected

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Fig. 7. Monthly environmental conditions during 1989: monthly mean upwelling index (Bakun 1973) centered at 45°N, 125°W (values >0 indicate upwelling; values <0 indicate downwelling); monthly mean index of vertical velocity into offshore Ekman layer (Bakun 1973) centered at 45°N, 125°W (values >0 indicate divergence; values <0 indicate convergence); monthly mean index of bottom temperature derived from Neah Bay, Washington, sea level height (Kruse and Huyer 1983) for two locations (55 m depth at 44°39′N, 124°11′W, and 100 m depth at 44°39′N, 124°27′W).

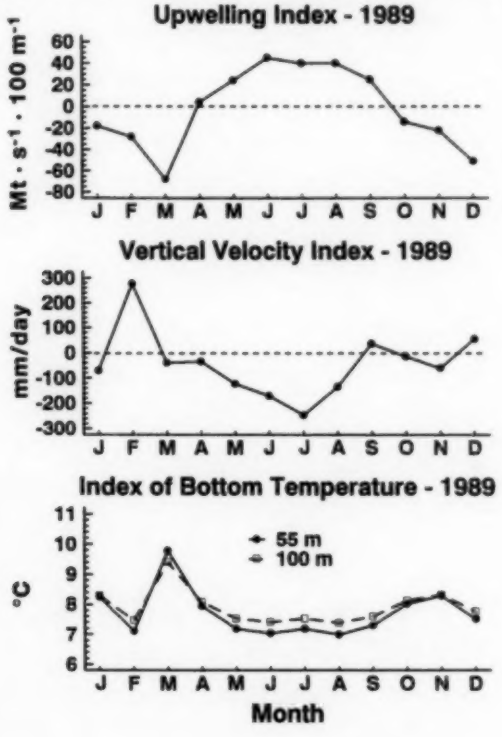


Fig. 8. Mean March transect (combined stratum) CPUE and 95% confidence limits of age-1 Dover sole (*Microstomus pacificus*) along the central transect, 1989–1994. Numbers of tows are indicated.

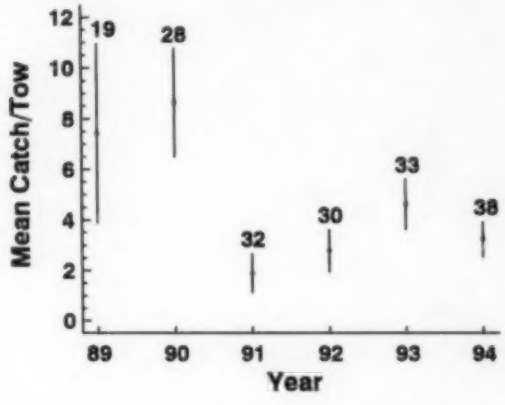
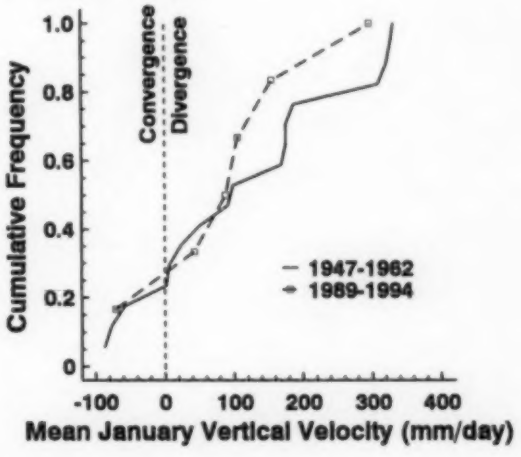


Fig. 9. Comparison of cumulative frequency distributions for mean index of January vertical velocity at 45°N, 125°W during 1947–1962 and 1989–1994.



at depths of 50–159 m had been on the bottom longer than larvae collected at 160–400 m. Larvae collected in deeper strata had been on the bottom an average of 17–20 days, whereas those in shallower strata had settled an average of 39–51 days prior to collection. This relationship also was reflected in the proportion of stage-3 larvae, the earliest demersal ontogenetic stage, at each station. The mean proportion was negatively correlated with mean number of days since settlement (days = 52.37 – 20.70 (proportion); $r^2 = 0.83$; $P = 0.033$).

The distribution of settlement dates was determined for each year between 1989 and 1993 (Fig. 13). Mean settlement dates ranged from 17 January to 5 February and differences among years were significant (equality of means test, $P = 0.013$). However, the only significant difference was between the 1991 and 1992 mean settlement dates. The early mean settlement date in 1992 coincided with a survey that was approximately 2 weeks earlier than the others. Neither mean settlement date ($P = 1.0$) nor mean days between settlement and collection ($P = 0.33$) were correlated with transect mean CPUE of age-1 Dover sole in March.

Discussion

Hayman and Tyler (1980) suggested that strong Dover sole year-classes resulted, in part, from the influence of an oceanographic process on settlement location. They proposed that, when winter downwelling is higher offshore than onshore, a zone of convergence restricts offshore waters and pelagic Dover sole larvae to the outer continental shelf. They suggested that settlement on the outer shelf provides a selective advantage related to food or physiological requirements immediately following settlement. Hayman and Tyler (1980) did not define onshore, offshore, or outer shelf. To facilitate comparison of our results with their hypothesis, we interpret the onshore–offshore scale of coastal divergence to be related to the baroclinic Rossby radius of deformation, which is approximately 10–20 km from shore near latitude 45°N (Bakun and Parrish 1980). This offshore distance corresponds to areas of approximately 60–100 m depth along the north transect,

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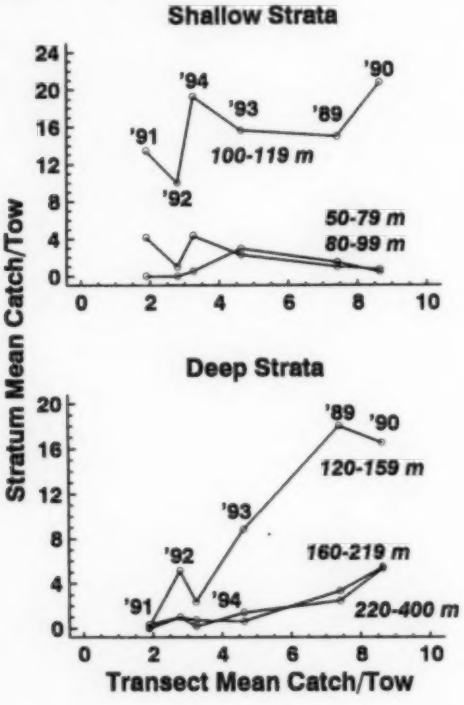
Fig. 10. Mean depth stratum CPUE of age-1 Dover sole (*Microstomus pacificus*) in relation to mean transect (combined depth strata) CPUE. Estimates are for March surveys on the central transect, 1989–1994.

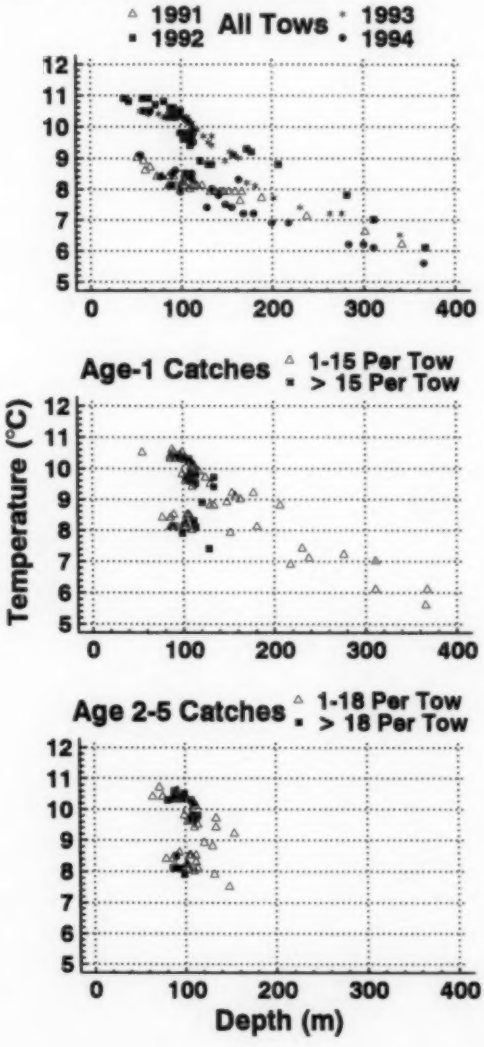


40–60 m depth along the central transect, and 50–60 m depth along the south transect (Toole 1994). We interpret the outer shelf as depths greater than 100 m (Hedgepeth 1957; Allen and Smith 1988).

Our results do not support the recruitment mechanism proposed by Hayman and Tyler (1980). Correlation between March age-1 CPUE and January offshore convergence would be expected if the hypothesis were correct. We could not demonstrate this correlation, despite a similar distribution of January mean vertical velocities observed during 1989–1994 and the years considered by Hayman and Tyler (1980) (Fig. 9) and despite significant contrast in annual age-1 CPUE (Fig. 8). A deeper distribution of age-1 larvae also would be expected to occur in years of offshore convergence. We found no correlations with depth distribution and November–January convergence and a significant correlation with February divergence, rather than convergence.

Several factors may account for our failure to confirm predictions of Hayman and Tyler's (1980) hypothesis: we have only 6 years of observations; the areas we sampled may not be representative of recruitment patterns throughout the entire statistical landing area considered by Hayman and Tyler (1980); cohort strength may be set after age 1; their cohort reconstructions may have been in error because they determined age from scales (Pikitch and Demory 1988); and, because Hayman and Tyler (1980) performed many simultaneous exploratory correlations that exceeded available degrees of freedom, this may have been a spurious correlation.

Fig. 11. Relation between depth and bottom temperature for individual tows on the central transect, 1991–1994. Tows corresponding to CPUE of at least one age-1 and age-2 to -5 (≤210 mm) Dover sole (*Microstomus pacificus*) are identified, as are the 20% of tows with highest CPUE.



Our data do support a key assumption of the Hayman and Tyler hypothesis: there is either a preference for, or a higher survival associated with, occupation of a bathymetrically restricted nursery area on the outer continental shelf during the first few months following settlement. In 5 of 6 years, the highest March age-1 CPUE on the central transect occurred in the 100- to 119-m stratum, with the next-highest catches occurring in adjacent 80- to 99-m and 120- to 159-m strata (Fig. 10). This distribution was not affected by an approximately 2°C change in bottom temperature between 1991 and 1994 (Fig. 11). During 1989, this depth distribution did not differ among three transects (Table 1) with different shelf widths and different depths of the inshore–offshore coastal divergence.

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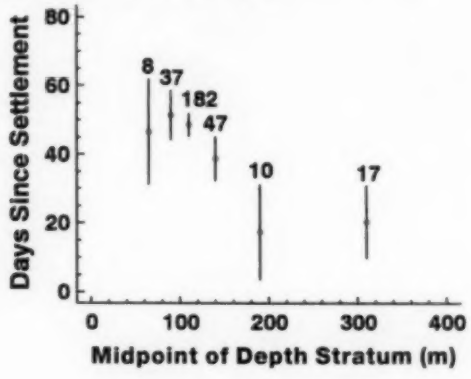
Fig. 12. Mean index of days since settlement, based on otolith increments distal to enclosure by accessory primordia (Toole et al. 1993), and 95% confidence intervals in relation to depth. Otoliths were from pooled subsamples of age-1 Dover sole (*Microstomus pacificus*) collected during March 1989–1993 on the central transect. Numbers of fish are indicated.



Our observations, coupled with those of Markle et al. (1992) and Butler et al. (1996), suggest that Dover sole larvae initially settle in deeper water and actively migrate inshore until the primary nursery area is found. Settling stage-3 and stage-4 Dover sole were caught from January to May (Fig. 3) over a broad range of depths (Fig. 5), temperatures (Fig. 11), and sediments. The distribution of days since initial settlement by depth (Fig. 12) and the greater proportion of stage-3 larvae in deeper water (the present study; Markle et al. 1992) support this interpretation. Stage-3 and stage-4 larvae are found both in midwater and on the bottom, suggesting that Dover sole transition from obligate midwater to obligate bottom inhabitants over several months (Markle et al. 1992; Butler et al. 1996). Markle et al. (1992) proposed that recently settled larvae continue to make nighttime vertical migrations, which would facilitate movement inshore during winter onshore Ekman transport (Parrish et al. 1981; Hickey 1989), during a process of continually testing the habitat in search of the preferred nursery ground.

The nearly complete absence of age-1 larvae between 50 and 79 m during the spring (Figs. 5 and 6) suggests an inshore distributional limit associated with relatively coarse sediment (mean median $\phi = 2.2$) and high bottom temperature (8.5–11°C). Because age-1 Dover sole move inshore to areas of coarser sediment and cooler temperature following the spring transition, it is unlikely that these physical features limit their initial distribution. We observed negative age–depth and size–depth relationships, which are opposite those reported for adult Dover sole (Hunter et al. 1990), both before and during the inshore migration. These relationships suggest faster growth or size-selective predation in the shallower parts of the spring nursery area. The assemblage immediately inshore of juvenile Dover sole is dominated by Pacific sanddab (*Citharichthys sordidus*) (Day and Pearcy 1968; Pearcy 1978; C.L. Toole, D.F. Markle, and C.J. Donohoe, unpublished observations), a species known to prey upon juvenile flatfishes (Wakefield 1984).

Although Dover sole appear to actively migrate inshore until encountering the spring nursery area, it is unclear which features of this habitat are important. Because temperature within the area of highest CPUE varied significantly among years (1991–1994), while its location did not, it is unlikely that temperature is a significant factor. Sediment type may be an important variable, but it grades continuously with depth in our transects, complicating interpretation. Pearcy (1978) was unable to detect an effect of sediment in an ANOVA predicting the number of juvenile Dover sole per tow off central Oregon. Pearcy (1978) sampled seven stations between 74 and 195 m, which included pairs with similar sediments (median ϕ ranging from 1.88 to 7.22) at different depths. He did not report the ages and lengths of Dover sole collected in that study, but cod-end mesh size (13 mm) was similar to that used in our study. Day and Pearcy (1968) and Pearcy (1978) also found that fish assemblages off Oregon correlated with depth, rather than sediment.

A combination of sediment and other factors may produce better feeding conditions for Dover sole near the 100-m isobath than in offshore areas. Food of Dover sole ≤100 mm consists of about 90% crustaceans and polychaetes and 10% mollusks during the winter months off Newport, Oregon (Pearcy and Hancock 1978). Within a 74–195 m depth range, the "edible biomass" of these prey groups available to juvenile Dover sole is greatest at 100–102 m depth (Bertrand 1971, cited in Pearcy and Hancock 1978).

The conditions that make the spring nursery area favorable for larval settlement may be influenced by the density of settlers. During high-abundance years, local density increased in strata deeper than 120 m but did not change in the 100- to 119-m nursery area or in shallower strata (Fig. 10). We were unable to detect a correlation between high CPUE and settlement timing, suggesting that the shift in distribution was not just an artifact of late settlement and incomplete migration to the primary nursery area. An increase of local density in marginal habitats during years of high abundance is commonly observed in fish populations and may be related to density-dependent habitat selection (MacCall 1990). Our study did not attempt to address a mechanism by which this might occur. A correlation with strong divergence in February was observed; however, we are unable to propose a reasonable explanation for this correlation and suspect that it may be spurious.

The conditions that make the spring nursery area favorable for settlement also appear to change seasonally. Following the spring transition, when upwelling begins, bottom temperatures decrease and Dover sole move to shallower depths. Demory (1971) and Pearcy (1978) also described this seasonal bathymetric migration. It may represent a response to higher primary productivity within 10–20 km of shore during the upwelling season (e.g., Fig. 1.25 of Landry et al. 1989) and the potential corresponding increase in demersal food production (Townsend and Cammen 1988). This seasonal migration may also be a mechanism for avoiding increasing numbers of predators in the spring nursery area during summer months. Alton (1972), who surveyed the northern Oregon coast using a large-mesh commercial bottom trawl, described the strongest seasonal fluctuations in catch rate between 91 and 137 m depth. Very low catch rates were observed during winter and spring, whereas catches two or three orders of magnitude higher occurred in the summer and fall. Pacific hake (*Merluccius productus*), "young" sablefish (*Anoplopoma fimbria*), and

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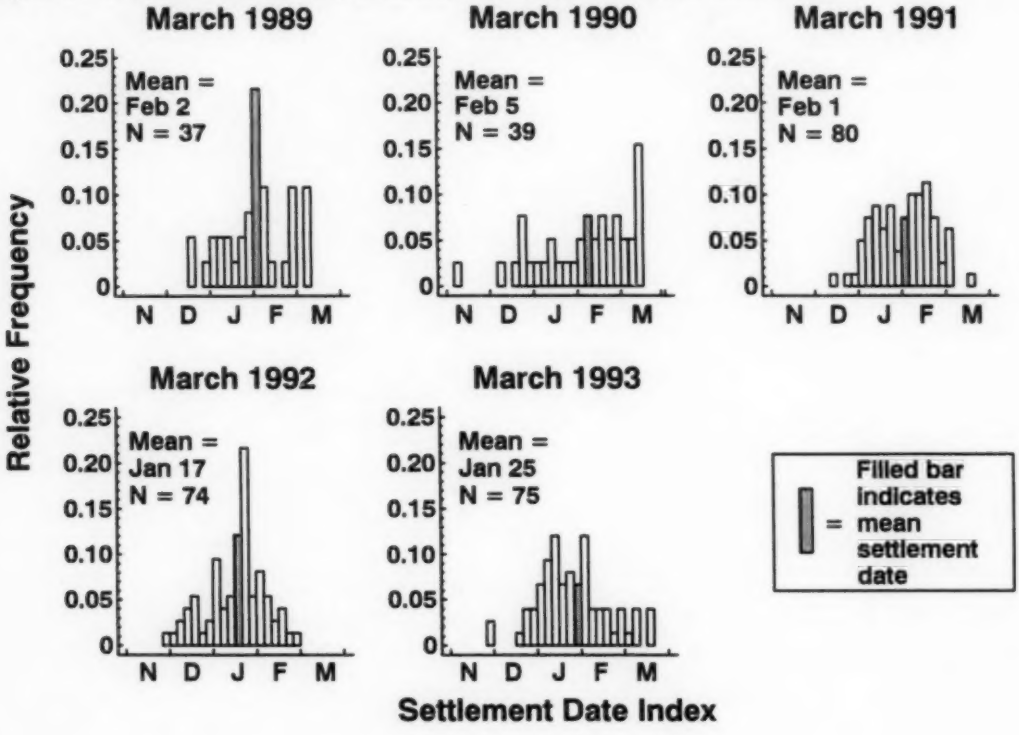
Fig. 13. Histograms of an index of days since settlement, based on otolith increments distal to enclosure by accessory primordia (Toole et al. 1993), for age-1 Dover sole (*Microstomus pacificus*). March 1989–1993 collections were on the central transect.



adult Dover sole accounted for most of the increase during summer months. Both Pacific hake (Rexstad and Pikitch 1986) and sablefish (Caillet et al. 1988) prey upon juvenile flatfish.

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Appendix

Number of valid bottom trawl samples by date, transect, and depth stratum.

Date and transect	50–79 m	80–99 m	100–119 m	120–159 m	160–219 m	220–400 m	Total
Jan. 1989 CEN	3	2	4	12	3	1	25
Jan. 1989 SOU	1	3	4	7	2	0	17
Jan. 1989 NOR	0	0	3	4	1	0	8
Mar. 1989 CEN	2	3	3	5	3	3	19
Mar. 1989 SOU	3	3	3	3	4	2	18
Mar. 1989 NOR	2	3	3	5	3	2	18
May 1989 CEN	3	3	3	3	3	2	17
May 1989 SOU	2	3	2	3	3	3	16
May 1989 NOR	3	3	3	3	3	3	18
July 1989 CEN	3	3	4	4	3	3	20
July 1989 SOU	3	3	3	3	3	0	15
July 1989 NOR	3	3	3	4	3	1	17
Sept. 1989 CEN	3	4	3	3	3	3	19
Sept. 1989 SOU	4	3	3	5	3	1	19
Sept. 1989 NOR	2	3	4	3	3	3	18
Nov. 1989 CEN	3	3	3	3	3	3	18
Nov. 1989 SOU	1	1	3	3	4	1	16
Nov. 1989 NOR	3	2	3	4	3	2	17
Mar. 1990 CEN	4	4	7	5	4	4	28
Mar. 1991 CEN	5	6	6	9	3	3	32
Mar. 1992 CEN	6	7	7	3	3	3	29
Mar. 1993 CEN	5	7	7	6	5	3	33
Mar. 1994 CEN	5	8	11	5	5	4	38
Total	69	80	95	105	73	50	475

Note: NOR, northern transect; CEN, central transect; SOU, southern transect.

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Production of Fraser River sockeye salmon (*Oncorhynchus nerka*) in relation to decadal-scale changes in the climate and the ocean

Richard J. Beamish, Chrys-Ellen M. Neville, and Alan J. Cass

Abstract: The abundance of Fraser River sockeye salmon (*Oncorhynchus nerka*) stocks was low in the 1960s, increased to high levels in the 1980s, and possibly entered a period of low abundance in recent years. The abundance changes of the combined stocks can be separated into productivity regimes that correspond to changes in climate trends. The most distinct change occurred when there was a major change in the climate over the Pacific Ocean in the winter of 1976–1977. The existence of natural shifts in abundance trends means that the high returns that occur during periods of high productivity would not be expected to occur during the low-productivity periods. The response of Fraser River sockeye to climate changes may be a specific example of a more general response by a number of species of fishes in the Pacific and perhaps in other oceans. Because the shift from one regime to the other occurred quickly in the 1970s, future shifts could also occur quickly. It is necessary to detect natural shifts in productivity when attempting to manage fishing impacts to ensure that economic expectations are sound and that overfishing does not occur.

Résumé : L'abondance des stocks de saumon rouge (*Oncorhynchus nerka*) du Fraser a été faible dans les années 1960, a atteint un niveau élevé dans les années 1980, et semble ces dernières années être entrée dans une période de faible niveau. Les changements observés dans l'abondance des stocks combinés peuvent être répartis selon des régimes de productivité correspondant à l'évolution des tendances climatiques. Le changement le plus distinct s'est manifesté lorsqu'on a observé une modification importante du climat au-dessus de l'océan Pacifique pendant l'hiver 1976–1977. L'existence de variations naturelles des tendances de l'abondance signifie que les fortes remontes observées pendant les périodes de grande productivité ne devraient pas se reproduire pendant les périodes où la productivité est faible. La réaction du saumon rouge du Fraser aux changements climatiques peut être un exemple spécifique d'une réaction plus générale présente chez un certains nombre de poissons du Pacifique et peut-être d'autres océans. Étant donné que le passage d'une régime à l'autre s'est produit rapidement dans les années 1970, d'autres changements tout aussi rapides pourraient avoir lieu dans l'avenir. Il est nécessaire de détecter ces changements naturels de la productivité si l'on veut tenter de gérer les effets de la pêche en misant sur des attentes économiques raisonnables et en veillant à éviter la surpêche.
[Traduit par la Rédaction]

Introduction

A major shift in the ocean–atmosphere system occurred in the North Pacific during the winter of 1976–1977 (Nitta and Yamada 1989; Trenberth 1990; Ebbesmeyer et al. 1991; Graham 1994). Large-scale atmospheric–oceanic changes resulted in an intensification of the Aleutian Low (the winter weather system in the subarctic Pacific), a drop in sea surface temperature (SST) in the central Pacific, and an increase in SST in the eastern Pacific (Venrick et al. 1987; Trenberth 1990). The shift in SST is most evident in the annual time series in the winter months. This is important because results of modeling indicate that winter is the period when the climate in the tropics has its maximal effect on the midlatitudes in the North Pacific (Miller et al. 1994). Late winter is also the time that many marine fishes in the subarctic Pacific reproduce. In the tropical Pacific, where the climate change was initiated (Kitoh 1991), there were dramatic changes in climate after the winter of 1976–1977 that have been linked to an intensification of the Aleutian Low pressure system in the subarctic Pacific (Niebauer 1988). In the tropical Pacific there were increases in SST, increases in westerly winds, increased convection, and an eastward shift in the region of organized convection (Graham 1994). The changes appeared to be similar to an El Niño but were generally viewed as a shift in background state of the climate (Graham 1994). The 1976–1977 change altered the pattern of the climate in the tropical Pacific until 1989, when it appeared that the pre-1976 pattern had been restored. However, in the 1990s, the persistent El-Niño-like conditions returned with an intensity that was unique in the time series (Trenberth and Hoar 1996).

Persistent biological changes in the subarctic Pacific after 1976–1977 included an increase in zooplankton abundance (Brodeur and Ware 1992; McFarlane and Beamish 1992; Beamish and Bouillon 1995), an increase in fish abundance in some areas (Ware and McFarlane 1989; Hollowed and Wooster 1992; Beamish and Bouillon 1993; Hare and Francis 1995; Polovina et al. 1994), and decreases in others (Ware and McFarlane 1989; Polovina et al. 1994). Pacific salmon (*Oncorhynchus* spp.) production almost tripled (Beamish and Bouillon 1991, 1993), producing historically high all-nation

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catch levels for several species of salmon from the late 1980s until the early 1990s. Catches of other important species also reached extremely high levels in the 1980s, and the abundance changes occurred over a relatively short time (Ebbesmeyer et al. 1991; Kawasaki 1991; Beamish 1993; Beamish and McFarlane 1995; Hare and Francis 1995). This evidence of persistent changes in abundance that occurred synchronously with large-scale shifts in the marine environment indicates that there are natural shifts in the long-term pattern of recruitment that we call regime shifts.

If climate-related productivity shifts are an important component of fisheries population dynamics, we should expect to see evidence of an impact of the major climate change in 1976–1977 on the productivity of the major fisheries in the Pacific. While there is evidence that catches of a number of species improved as a result of the 1976–1977 change, it has been difficult to study the productivity trends of important stocks before and after 1976–1977 because it is rare to have a reliable long-term time series of measurements of the spawning biomass and the subsequent production from this spawning. One of the best series of fish production data in the North Pacific is for Fraser River sockeye salmon stocks. The data are reliable because the stocks are fished by Americans and Canadians under a sophisticated international management system. The numbers of spawning fish (escapement) and the resulting catch from each spawning were estimated, initially by the International Pacific Salmon Fishery Commission and recently by its successor, the Pacific Salmon Commission. Since 1985, the Department of Fisheries and Oceans (Canada) has estimated escapements. The use of catch and escapement avoids the difficulties of attempting to use only catch as a measure of production as was required in the studies of Beamish and Bouillon (1993) and Hare and Francis (1995).

The objective of this study was to determine if the climate changes in 1976–1977 could be shown to affect the productivity of Fraser River sockeye salmon, that is, to determine if there was evidence of distinct productivity periods before and after 1976–1977. Sockeye salmon in the Fraser River spawn in the fall, with the year of spawning termed the brood year. Early in the year after spawning, most sockeye fry emerge from the gravel and move into lakes, where most remain for 1 year. Toward the end of April of the following year, the young sockeye enter the ocean as smolts and quickly move out of the nearshore areas into the high seas. Approximately 93% of the sockeye salmon from the Fraser River spend 2 years in the ocean before they return to spawn (Roos 1991). There are 8 major stocks of sockeye salmon and over 50 spawning areas in the Fraser River. Some of these major stocks, which return to the Fraser River at different times throughout the summer, exhibit distinct 4-year cycles in abundance.

Methods

The production regime for sockeye salmon after the 1976–1977 ocean–climate change was considered to begin for the brood year of sockeye salmon that entered the ocean in the spring–summer of 1977 (i.e., the 1975 brood year). We terminated this regime after the 1989 brood year or the 1991 year to sea because there is evidence of a climate–ocean change that could affect the marine survival of Fraser River sockeye in the mid-1990s (Fig. 1; Roemmich and McGowan 1995; Trenberth and Hoar 1996). Figure 1 is a modification of the Aleutian Low Pressure Index described in Beamish and Bouillon (1993). This modified index is for December through to the end of March with the year referring to January in this 4-month series. The anomaly for each year is the average monthly value for the 4-month period subtracted from the average monthly value for the time series. The smoothed trend indicates the general pattern and is virtually identical ($R^2 = 0.82$) to the smoothed trend shown in Beamish and Bouillon (1993) and to the inverse of the Aleutian Low pressure index developed by Trenberth and Hurrell (1995). We include the 1990 and 1991 brood year data (the 1992 and 1993 year to sea) in the time series for comparison, as it is possible that the change in the Aleutian Low in the early 1990s signals another change in the productivity trend that started with these brood years. The 1989, 1990, and 1991 brood years do not include fish older than age 4, which represent approximately 7% of the returns as previously mentioned. Selecting the start of the time series for the regime prior to 1977 was less clear. There was a brief period of intensification of the Aleutian Low in the mid-1950s (Fig. 1; Leathers and Palecki 1992; Beamish and Bouillon 1993), and there was a strong El Niño in 1957–1958 (Quinn and Neal 1987). The regime prior to 1977 would have to have begun when the Aleutian Low weakened, or before the intense low in 1959 (Fig. 1). Thus, we started our time series in 1958, i.e., for smolts that went to sea in 1960. By starting in 1958, we excluded 9 years of data, as reliable stock-specific recruitment data began in 1948 (Roos 1991).

We compared stock-recruitment curves and the pattern of residuals from these curves to indicate the effects of the environment on survival. The selection of a stock-recruitment relationship was somewhat arbitrary because of the variability in the data. For most relationships we used the standard Ricker curve, $R = \alpha P e^{-\beta P}$ (where R is the number of recruits, P is the size of the parental stock, α is a parameter that represents density-independent factors, and β represents density-dependent factors; Ricker 1975), which is commonly used for Fraser River sockeye salmon. Residuals are the deviations from the relationship $\ln(R/P)$ versus P. The escapement data used in this study, unless otherwise indicated, are estimates of females that contribute to spawning and not the total number of males and females estimated to arrive at the spawning area (Gilhousen 1990). This estimate is commonly used to indicate spawning potential because it is an estimate of the proportion of females that fully contribute to spawning based on estimates of the number of females entering the spawning grounds and egg deposition, i.e., the number of females weighted by the percentage of eggs released. The term frequently used to describe this estimate is effective females; however, in this report we refer to this estimate as spawning females. A comparison of the consequences of using total spawners and spawning females to estimate escapement is included in Fig. 2. Because we are studying ocean effects on the survival, the annual estimate of spawning females is recorded in the figures as the year the offspring enter the ocean unless indicated. The spawning or brood year is 2 years earlier as mentioned (Roos 1991). A reference to brood years will also be used, as this is a common way of identifying salmon year-classes.

An alternative stock-recruitment analysis was performed that gives equal weight to each of the major stocks (early Stuart, late Stuart, Stellako, Chilko, Quesnel, Birkenhead and Seymour, late Shuswap). The purpose of this analysis was to reduce stock-specific effects on survival. Residuals from a Ricker curve fit to each stock were standardized by subtracting the 1958–1989 or the 1958–1991 brood year stock-specific mean residual and then dividing by the corresponding stock-specific standard deviation. Residuals for combined stocks were computed as the mean of the standardized residuals in each year. Estimates of R/P (recruits per spawner) resulted patterns similar to those of residuals from recruitment curves (data not shown). Artificially enhanced stocks and small stocks where data quality was poor were excluded.

In addition to the total returns of all stocks we used two of the largest stocks or stock complexes, the Chilko Lake stock, and the late run of the Adams River stock as examples of stock specific productivity relationships. The late-run Adams River stock, which we will

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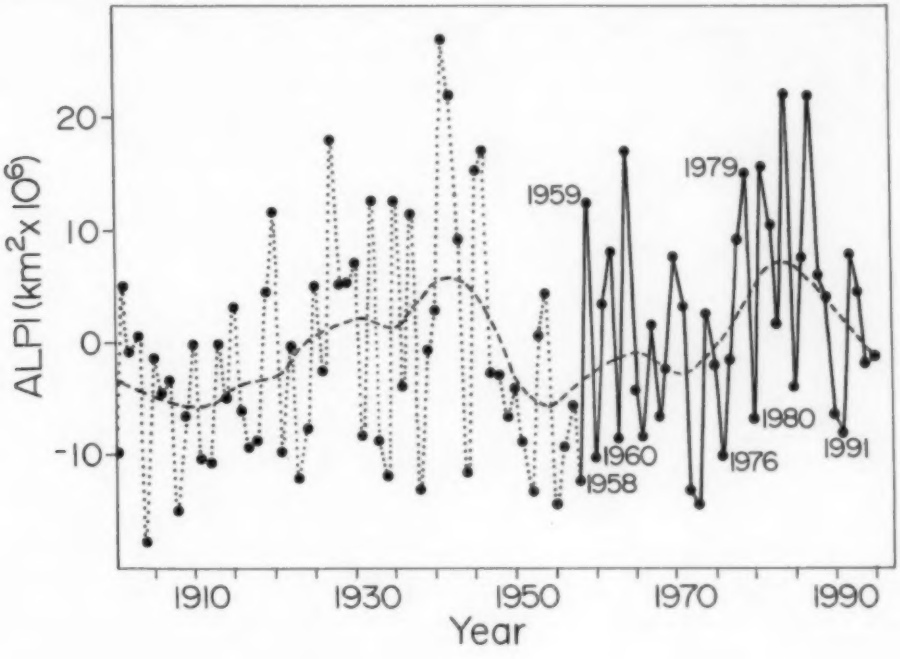
Fig. 1. The 4-month averaged Aleutian Low Pressure Index (ALPI) anomaly, modified from Beamish and Bouillon (1993). For each month from December, January, February, and March, the average area of the ocean covered by the low less than 100.5 kPa was estimated. The average for the 4 months was subtracted from the series average and the resulting anomaly was used as the yearly index for the January year. The solid lines indicate the time series used in this study and the broken line is the loess smoothed line (band width, *f*, of 0.20). The years beside some annual values relate to specific issues in the text.



refer to as the late Shuswap stock, includes estimates of several stocks that spawn in the tributaries of Shuswap Lake and consistently have been the largest producer of sockeye on the 1990, 1994 cycle line. Another major stock not included in this analysis is the Quesnel Lake sockeye stock. Ricker stock-recruitment parameter estimates for β were negative as a result of exponential population growth on the dominant cycle line (1993, 1989, 1985, . . .) in the 1980s. For that reason we chose to exclude Quesnel sockeye, although increases in their survival in the 1980s were the highest of any Fraser River population.

We examined marine survival trends using estimates of smolts leaving Chilko Lake. Because the smolts were leaving the lake and would enter the ocean directly, these data provide the most accurate measurement of marine survival available. These estimates are the only reliable smolt production data that are available for the complete time series used in this study.

The differences in stock-recruitment relationships between the two regimes for Chilko Lake sockeye smolts were examined using straight line fits and a Beverton–Holt relationship because adult abundances in the second regime showed no evidence of decline at high smolt production. A statistical power analysis (Peterman 1990) was used to assess the probability of detecting a long-term change in the productivity parameter, α, given the large interannual variation (noise) in the stock-recruitment relationship. We simulated 500 smolt–adult recruitment data sets using a Beverton–Holt recruitment function (Schnute 1985, eq. 1.27) at increasing increments of α to simulate increases in survival rates (returns/smolts) ranging from 30 to 100%. The parameter α was estimated from the 1958–1974 relationships between smolts and adult returns. The stochastic noise in each of the 500 simulated data sets was fixed at the residual standard deviation estimated from the 1958–1974 brood year relationship. For each simulation, the time series of annual smolt abundance estimates observed in 1975–1988 (14 years) was used to generate subsequent returns. We used a simplex search method (Nedler and Mead 1965; Mittertreiner and Schnute 1985) to compute the joint α–β parameter 95% confidence regions (Edwards 1972) for the observed (real) data. For each increment of α, the distribution of 500 α–β parameter estimates were compared with the joint α–β 95% confidence region for the 1958–1974 smolt-return data.

The relationship between fishing and productivity changes was examined using harvest rates (total catch/total return of age-4 fish). The trend in harvest rates was compared with the total return and with the total escapement (not spawning females) so that all fish were included. All data were plotted against brood year to simplify the comparison of harvest rate with returns and escapement.

The changes in productivity were also compared with El Niño events. To make the comparison, we determined the total return per spawning female for each year and calculated the yearly anomaly from the average of the entire time series. The yearly anomalies were plotted as the year the smolts from the spawning females went to sea. A positive (+) anomaly identifies years when adult production was good, and a negative (–) anomaly identifies years of below average production. El Niño years were identified using the data from Enfield (1989) and Barnston et al. (1994).

We compared total Fraser River sockeye salmon returns with the combined catches of sockeye salmon from the United States, Canada (minus Fraser River catch), Russia, and Japan to determine if there was a synchrony in the response to changes in the ocean environment. Age-1.2 (one freshwater annulus and two saltwater annuli or equivalent to age 4) sockeye are the most common in the combined all-nation fisheries, but older sockeye exist. Although it would be more accurate to compare total returns by adding up the ages in the catches to produce an accurate account of brood year production, this level of

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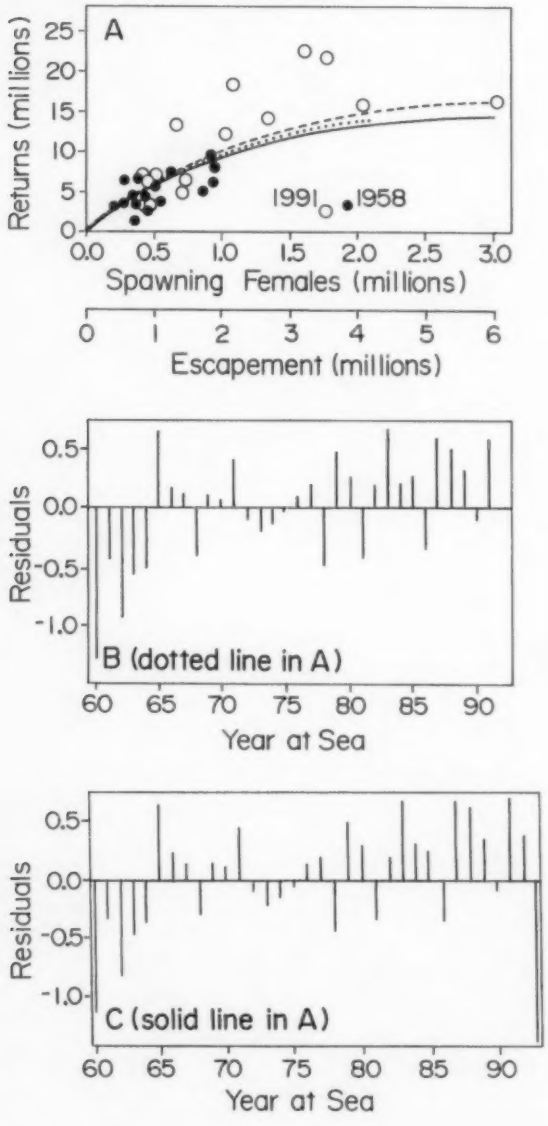
Fig. 2. (A) The escapement of spawning female sockeye salmon in the Fraser River and the subsequent total return for brood years 1958–1989 (dotted line, $R = 13.33Pe^{-0.343 \times 10^{-6} P}$) and 1958–1991 (solid line, $R = 13.66Pe^{-0.419 \times 10^{-6} P}$). The two brood years with exceptionally low returns (1958, 1991) are identified separately. The solid circles are the years from 1960 to 1976 year to sea (or 1958–1974 brood year), and the open circles are from either the 1977–1991 or the 1977–1993 year to sea (or 1975–1989 or 1975–1991 brood year). The dashed line is the relationship using total escapement instead of spawning females. (B) The residuals from the dotted line in A. (C) The residuals from the solid line in A. Residuals are deviations from $\ln(R/P)$ versus P.



detail is not available for the all-nation catches. Our comparisons, therefore, provide a general indication of the relationships between Fraser River production and all-nation minus Fraser River catch.

Results

The relationship between the total returns for all sockeye salmon stocks, for the brood years 1958–1989, in relation to the spawning female escapements that produced these returns (Fig. 2A, dotted line) showed a trend in the residuals of the stock and recruitment relationship (Fig. 2B). The slope of the residuals was different from zero (t test, $p < 0.01$, $R^2 = 0.31$), confirming that the trend was not random. There was also a trend in the residuals to the Ricker curve when the time series extended to the 1991 brood year, i.e., 1993 year to sea (Fig. 2C, solid line; t test, $p < 0.01$, $R^2 = 0.31$). The use of total escapement and estimates of spawning females produced virtually identical relationships (Fig. 2A, dashed line).

An intervention analysis (Noakes 1986) using the series from 1958 to 1989 brood years indicated that there was a significant step up in the time series of total returns in 1977 (step intervention = 2.70×10^6, SE = 1.27×10^6, $p = 0.05$; Fig. 3). Thus, our separation of the time series between the 1976 and 1977 catch years (1974 and 1975 brood year) was consistent with both the biological and physical data. Separating the production data for brood years 1958–1989 into the two productivity regimes produced two very different stock-recruitment relationships (Fig. 4A). The maximal production in the higher productivity regime determined from the Ricker curve was approximately 3.5 times greater than in the lower productivity regime (Fig. 4A). A loess fit (Cleveland 1985) of the total brood year returns for each regime indicated that there was a small increasing trend in the returns in the early regime and a large increase in the trend in the later regime (data not shown); thus, the two regimes were characterized by a period of gradually increasing production followed by a period of steep increases. The pattern of residuals for the early regime (year to sea, 1960–1976) still had a trend in the residuals (Fig. 4A; t test, $p < 0.05$, $R^2 = 0.27$), but the year to sea from 1977 to 1991 (1975–1989 brood year) had no trend in the residuals (Fig. 4C; t test, $p > 0.05$, $R^2 = 0.01$).

The mean standardized residual pattern for the eight major stocks was marginally not significant (t test, $p = 0.06$) for the series from 1960 to 1991 year to sea (Fig. 5A). The absence of a significant trend at a $p = 0.05$ level but the presence of a significant trend at a slightly higher level of $p = 0.1$ is an indication that there was a trend or pattern in the mean standardized residuals for the eight major stocks. There was a tendency for residuals to alternate from a negative trend at the beginning of the time series for a period of 5 years (1960–1964) to a period of generally positive anomalies for 11 years (1965–1975). This was followed by a period where the trend was from negative to positive (1976–1991). As mentioned, the most recent change appears to have occurred with the 1992 and 1993 year to sea (Fig. 5B).

There was virtually no difference in the shape of the Ricker curves for the Chilko Lake stock for either of the two regimes (Fig. 6A). As would be expected, there also was not a significant trend in the residuals (Fig. 6B, t test, $p > 0.05$). There was, however, greater variation in the returns in the second regime (Fig. 6A, open circles) than in the first regime (solid circles). The Ricker curves for the late Shuswap stock differed among regimes and from the curve for the total time series (Fig. 7A). It is clear from Fig. 6A that the 1958 brood year data point had a major impact on the shape of the curves. Despite the difference

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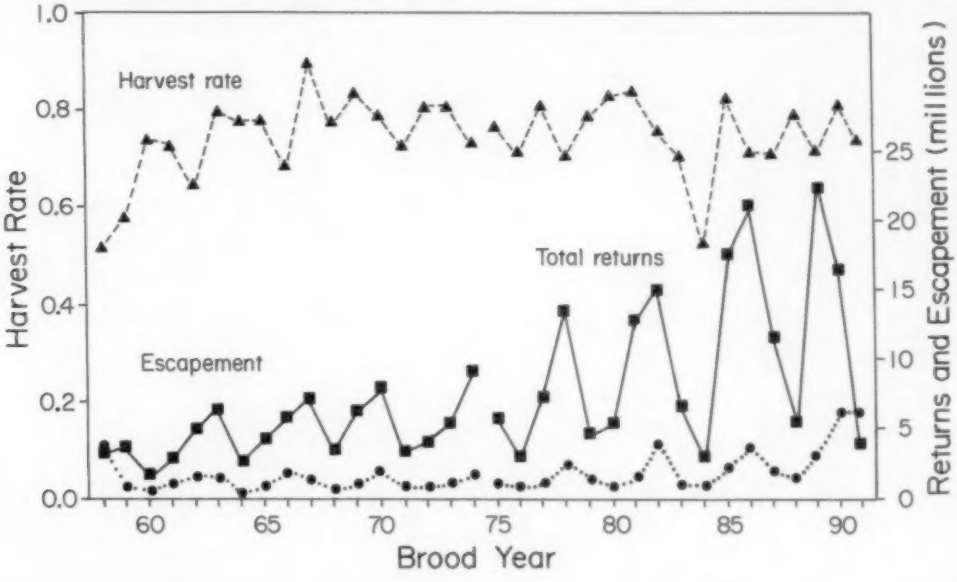
Fig. 3. The harvest rate, total returns, and escapement for the combined Fraser River stocks in relation to the brood year that produced the return. The brood year is identified, and the total returns are lagged back to the brood year that produced the return. For any brood year, the total return is for ages 4 and 5. The harvest rates for each brood year are for age-4 fish only. As described in the text, 93% of the returns occur 4 years later than the brood year. The escapement is not lagged and indicates the year of spawning. The data are separated at the 1974 brood year to identify the regimes before and after the 1976–1977 climate event.



in the shape of the Ricker curves, there was no significant trend in the residuals (t test, $p > 0.05$; Fig. 7B).

A comparison of counts of smolts migrating to the ocean from the Chilko Lake stock showed distinct relationships for the two regimes (Figs. 8A and 8B). The returns from the years of larger smolt production were up to two times greater for the same number of smolts produced in the period from 1977 to the 1990 year to sea than in the earlier regime. Although straight-line fits through the origin for both regimes may not be the most appropriate relationship theoretically, the straight line fits do facilitate a comparison of the two data sets. The slope of the data in the later regime was different from the slope of the data in the earlier regime (ANOVA, $p < 0.05$), indicating a significantly higher survival.

It is apparent that neither the straight line nor the Beverton–Holt relationships adequately described the data in the second regime. However, as seen in Fig. 9, the ellipses formed by the joint Beverton–Holt parameters α–β 95% confidence intervals overlapped and did not show a distinction between recruitment rates of the two regimes.

On the basis of the smolt–adult recruitment simulations, the power of detecting a long-term (14 year) change in survival rate was poor at survival increments <70%. Only at a survival increase of ≥70% was there a 95% probability of detecting a 14-year change in survival, given the inherently noisy stock recruitment data. On the basis of these simulations, it is unlikely that 14- to 18-year regime shifts in Fraser River sockeye survival can be detected by comparing stock–recruitment curves, unless the change in survival is very large for most years, or the duration of the regime is persistently longer that reported here.

There was no difference (t test, $p < 0.05$) in the average harvest rate of 74.1% (Fig. 3) in the early regime (brood years 1958–1974, years to sea 1960–1976) compared with 74.6% in the regime from 1977 to 1991 (brood years 1975–1989). The harvest rate did not include age-5 returns, but as they averaged approximately 7% of the returns, the estimated harvest rates were considered to be representative. Despite the rather constant harvest rate, the total returns increased in the second regime. There were some years of higher escapement in the regime after 1976 compared with the early period. The escapement data series exhibited a 4-year cycle, and there was a trend in the residuals from the series mean. When the log-transformed escapement data for spawning females was tested for changes in the mean for the regimes before and after the 1976–1977 climate shift, using an intervention analysis, there was no significant difference in the two means ($p > 0.05$) for series extending to either the 1991 or 1993 year to sea.

We also examined the impact of escapements on production trends by comparing residuals with escapement for both the combined stock data and the eight major stocks time series. The escapement for the combined stocks was the estimate of spawning females and for the eight major stocks; the escapement was the summed spawning female estimate for these stocks. In both examples (Figs. 10A and 10B) the relationship between escapement and residuals was not significant (t test, $p > 0.05$) for the series either from 1960 to 1991 or from 1960 to 1993 (brood year 1958 to 1989 or 1991), indicating that the increasing trend in residuals (i.e., survival) in the 1980s cannot be readily related to increasing escapements.

From 1960 to 1993, there were seven El Niño events (Fig. 11). Fraser River sockeye production from smolts that went to sea in three of these events (1965–1966, 1969–1970, 1982–1983) was higher than average; in two, production was

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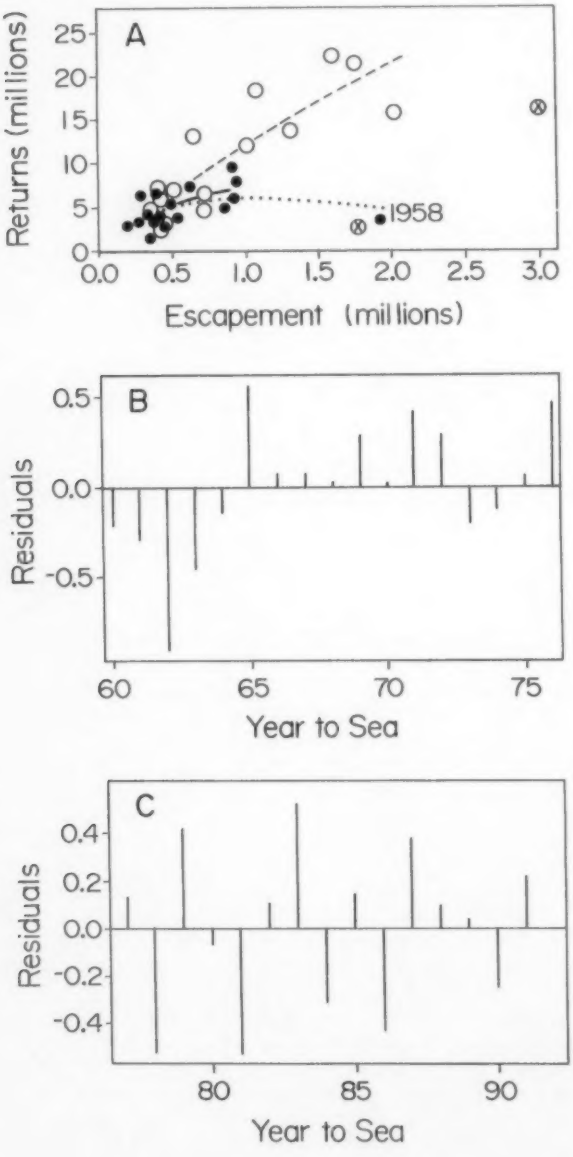
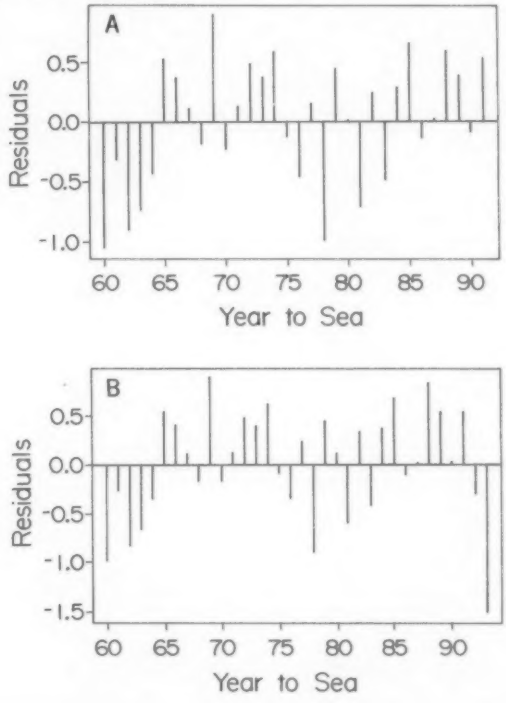
Fig. 4. (A) The escapement of sockeye salmon to the Fraser River and the subsequent total returns for two regimes, 1960–1976 (solid circles, dotted line, $R = 17.28Pe^{-1.052 \times 10^{-6}P}$), and 1977–1991 (open circles, dashed line, $R = 12.70Pe^{-0.0746 \times 10^{-6}P}$). The short line is the relationship from 1961 to 1976, i.e., without the 1958 brood year. The 1990 and 1991 brood year points are identified as an open circle with an × but are not used in the calculation of the Ricker curves. (B) The residuals from the Ricker curve for the 1960–1976 regime. (C) The residuals from the Ricker curve for the 1977–1991 regime.



lower (1972–1973, 1992–1993); and in two events, both above and below average production rates were recorded (1977–1978, 1986–1987). Thus, there was not a consistent relationship between sockeye production and El Niño events.

Fig. 5. (A) Mean standardized residual pattern for the eight major stocks showing the tendency for the pattern to be grouped into three periods: 1960–1964, 1965–1975, and 1976–1991. (B) The same pattern as in A, but extending the series to include the 1990 and 1991 brood years (1992 and 1993 year to sea) to show the possible early signs of a new trend.



The relationship between Fraser River sockeye production and all-nation catch minus Fraser River catch indicates that reduced catches in the early regime occurred throughout the North Pacific followed by synchronous increases in sockeye salmon catches in the recent regime. The 1990 (Fig. 12A) cycle line (i.e., 1990, 1986, 1982, . . .; sometimes called the Adams River cycle) and the 1989 (Fig. 12D) cycle line (i.e., 1989, 1985, 1981, . . .) were the most abundant of the four cycles in the Fraser River. For both of these cycles there was no indication of years of high production in either the Fraser River stocks or all-nation minus Fraser River catch in the low productivity regime, but there was an increasing linear trend in the high-productivity regime (Figs. 12A and 12D). The increasing returns to the Fraser River in the high-productivity regime were clearly associated with increasing production of sockeye throughout their range. The 1988 cycle line (i.e., 1988, 1984, 1980, . . .) and the 1987 cycle line (i.e., 1987, 1983, 1979, . . .) were weaker cycles, but the relationship with all-nation catch still appeared as two distinct regimes (Figs. 12B and 12C). As the all-nation minus Fraser River catch increased, there was a corresponding increase in returns to the Fraser River for both these cycles (except for 1979).

Discussion

The intervention analysis identified a significant shift in

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Fig. 6. (A) The escapement of sockeye salmon for the Chilko Lake stock and the subsequent total return (solid line). The data stop at 1990 (the 1988 brood year) because the lake was fertilized after this brood year to improve freshwater production. The solid circles are the years from 1960 to 1976 (dotted line), and the open circles are from 1977 to 1990 (dashed line). (B) The residuals from the relationship from 1960 to 1990.

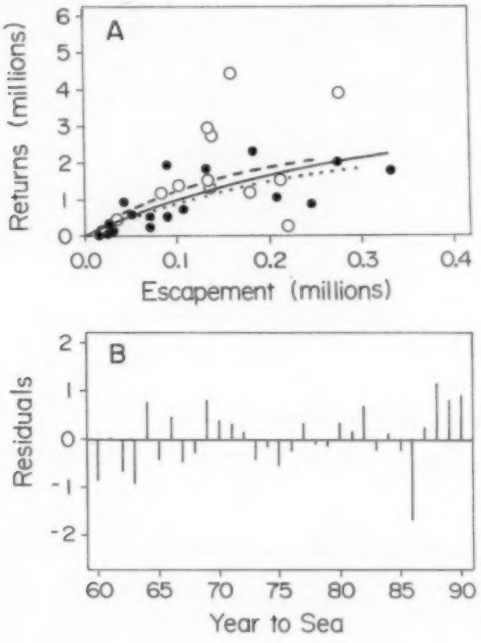
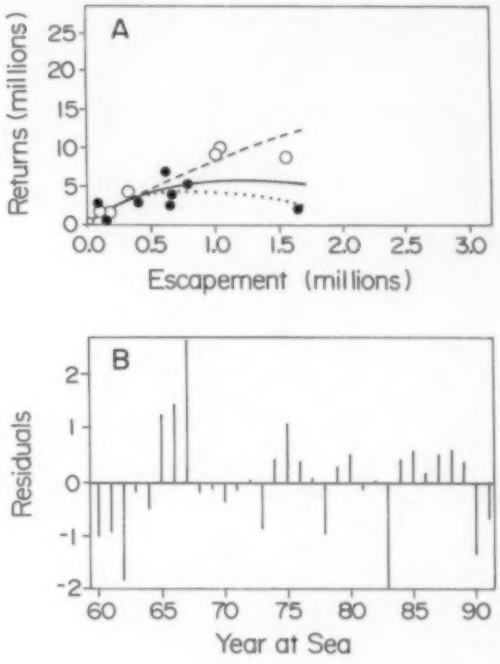


Fig. 7. (A) The escapement of sockeye salmon for the late Adams River stock and the subsequent total return (solid line). The solid circles are the years from 1960 to 1976 (dotted line), and the open circles are from 1977 to 1992 (solid line). (B) The residuals from 1960 to 1992. Note that the escapement for 1958 is for one stock and is lower than in Fig. 3A.



abundance in the Fraser River sockeye stocks in 1976–1977, as has been reported for sockeye production in Alaska (Hare and Francis 1995). In Alaska and in the Fraser River, the change in abundance before and after 1976–1977 was associated with the large-scale change in climate as previously mentioned. The weakening of the Aleutian Low in the early 1990s and the poor returns in 1994 and 1995 may be an indication that a new trend in production has started for Fraser River sockeye.

There was clear evidence of distinct productivity regimes when the total returns were compared with the escapements that produced these returns. The stock-recruitment curves for the total returns for all stocks in the Fraser River are different between the two regimes and convey quite different information about productivity when compared with the stock-recruitment curve that combines the two regimes. For example, there is no indication from the stock-recruitment curve for the early or low-productivity regime that large increases in escapements would have produced large returns similar to those observed in the 1980s. There also was evidence from the smolt production data from Chilko Lake that the marine survivals were higher in the years following the 1976–1977 climate change. Because the smolt estimates were made when the smolts left the lake to migrate to the ocean, the difference in productivity in the two periods could only result from differences in the survival in the ocean or during the approximately 430-km journey from Chilko Lake to the ocean. The differences in Chilko Lake smolt production between the two periods were less clear when examined using a stock-recruitment model. However, because of the high interannual variability, the power of detection is low as shown by our simulation study. A rather striking example of the impact of the regime shift in 1976–1977 on Fraser River sockeye salmon stocks was identified by Cox (1997). In the regime prior to the shift the marine growth of fish from the major sockeye stocks in the Fraser River did not exhibit a trend. However, from 1978 to 1992 these stocks showed a significant negative trend in marine growth.

There also was evidence that the increases in Fraser River sockeye production occurred at the same time as increases in sockeye production throughout the subarctic Pacific. Synchronous increases over such a large area would most likely result from changes in the marine survival. As mentioned, other salmon species and many other fishes increased in abundance at this time (Beamish and Bouillon 1993, 1995; Kawasaki 1991; Shuntov et al. 1993). The dramatic increase in fish production in the late 1980s in the subarctic Pacific is a clear indication of a major increase in ocean productivity. It is most probable that, if the productivity of a large number of species increased because of high ocean productivity levels, the increases in Fraser River sockeye abundance was also related to the improved ocean productivity.

The evidence of distinct productivity regimes for Fraser River sockeye was less clear when individual stocks were studied. Some differences in the stock-recruitment relationships of individual stocks may occur because the estimates of returns

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Fig. 8. The total return from the actual number of smolts estimated to have left Chilko Lake during the migration to sea, separated into (A) 1960–1976 and (B) 1977–1990.

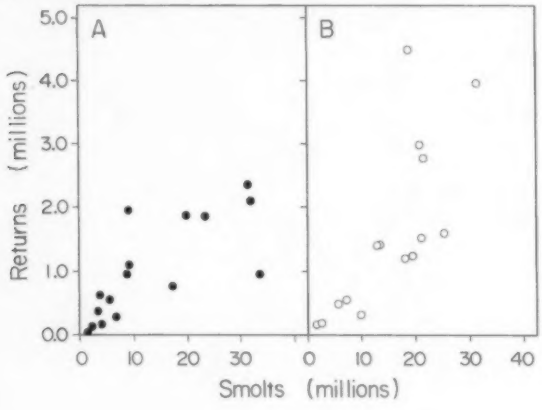
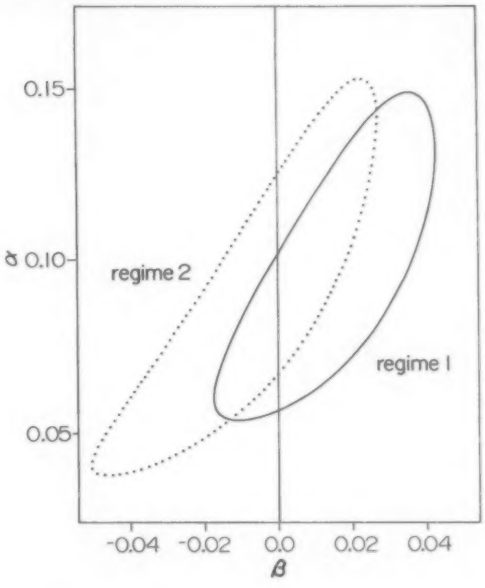


Fig. 9. Joint 95% confidence intervals for the Ricker stock-recruitment parameters.



at the stock level are less accurate than the total estimates of returns as a consequence of errors associated with the method used to partition total catches into stocks (Cass and Wood 1994). There may be stock-specific freshwater and marine effects that are less influential when the data from all stocks are combined. It also is important to note that if accurate smolt counts were available rather than estimates of spawning, it would be possible to study the stock-specific effects in more detail. The standardized estimate of residuals for the eight major stocks and the two specific examples did not show periods of productivity as distinct as those found for total stocks (except perhaps in the two most recent years, 1992 and 1993 year to sea). The eight major stocks (and the total stocks) did exhibit a pattern of production that was similar to the pattern observed for the combined stocks, suggesting coherence among years and changes from one pattern to another. In addition to the change before and after 1976, both the eight-stock series and the all-stock time series had a change in the trend of the residuals in the 1960s. The increased intensity of the Aleutian Low from 1959 to 1964 may account for the change in the residual pattern beginning with the smolts that went to sea in 1965. The generally less intense lows from 1966 to 1976 corresponded to the change in trends from the late 1960s to the late 1970s when the impacts of the 1976–1977 change became apparent. The change again in the early 1990s in the Aleutian Low Pressure Index corresponds with the low returns of smolts that went to sea in 1992 and 1993. Thus, while we were studying the effects of the 1976–1977 regime change, there appear to be other factors that affected production in the period from 1960 to 1976, and again in the mid-1990s.

Another explanation for the large increases in production in the 1980s would relate to changes in escapements or harvest rates. Again, there was no clear evidence that linked such changes to the improved returns. In fact, the harvest rates in the 1980s were slightly higher than in the 1960s. In particular, the late Adams River stocks had reduced harvest rates from 1960 to 1970 compared with the 1980s (Cass and Wood 1994). There also was a very short fishing season in 1963 as a result of a strike that resulted in anomalously low harvest rates. Despite this reduced fishing pressure, the returns in 1967 were similar to the returns in 1963. Thus, there is no apparent trend in the pattern of fishing that could explain the changes in productivity between the two regimes.

As mentioned, there were increased escapements during the second regime, and undoubtedly they contributed to the increased production through the increases in marine survival. Even though we did not identify a significant increase in escapements, we would expect that the increase in returns is in part related to these improved escapements, assuming that the increase in escapements produced an increase in the numbers of juvenile sockeye salmon entering the ocean. The improved marine survival would also lead to increased returns, facilitating management decisions to increase escapements. We think that both the increase in marine productivity and the management practices in the 1980s resulted in the improved returns, but the magnitude of the increases would not have been possible without the regime change.

It is not a new conclusion when examining fisheries data to discover that interpretations are not clear. Our interpretation of these data is that distinct productivity regimes do exist and that there was an average increase in productivity after the winter of 1976–1977. The change did not represent an incremental increase in all stocks in all years, rather the environmental conditions (intense Aleutian Lows) that produced higher marine survival were more frequent in the second regime, resulting in a general increase in productivity. The Chilko smolt data indicated that the increased survival seemed to occur for the years of higher smolt production or larger escapements. There were 4 years when the marine survival was exceptionally high in the second regime and there was nothing equivalent in the early regime. It was apparent from the data that the highest levels of smolt production in the early regime did not result in returns as large as those seen in the 1980s, whereas similar high levels in the recent regime did.

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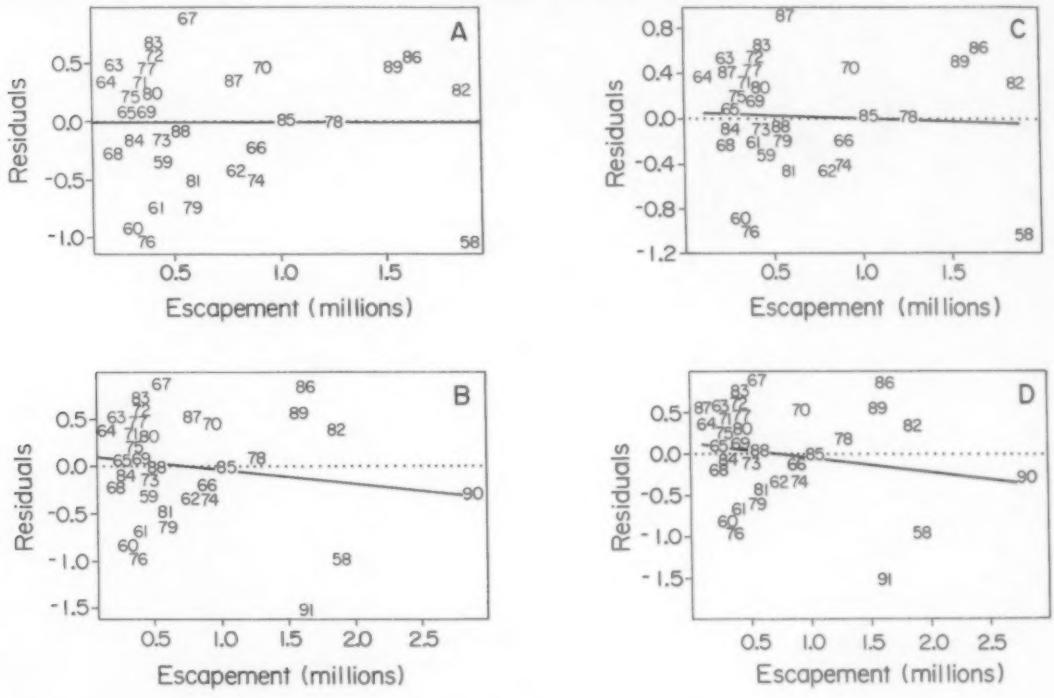
Fig. 10. Trends in residuals from the Ricker curve for the combined stocks compared with the escapement of spawning females for the series for the (A) 1958–1989 brood year (1960–1991 year to sea) and (B) 1958–1991 brood year (1960–1993 year to sea) and for the average standardized residual for the eight stocks for the (C) 1958–1989 brood year (1960–1991 year to sea) and (D) 1958–1991 brood year (1960–1993 year to sea). The solid lines are the trends in escapements. All years are brood years.



There may be some question about the use of the 1958 brood year in the early time series. We used the 1958 data point because it fell within the criteria used for the identification of the early regime. In 1958, a surplus escapement to the Adams River occurred after October 7 because disagreements within the fishing industry prevented the harvest of these fish (International Pacific Salmon Fisheries Commission 1959). The International Pacific Salmon Fisheries Commission subsequently prevented surplus fish from entering the Adams River to spawn. The fish that were prevented from spawning in the river spawned on the beaches of a lake where egg to fry survival was believed to be poor (International Pacific Salmon Fisheries Commission 1959). It was also noted that survival up to hatching in the river was excellent (International Pacific Salmon Fisheries Commission 1959). The issue is whether low returns from the 1958 spawning were a result of high natural mortality after the egg to fry stage or the manipulations by the International Pacific Salmon Fisheries Commission, or both. In the opinion of the International Pacific Salmon Fisheries Commission, the poor return was a result of the poor marine survival (International Pacific Salmon Fisheries Commission 1963). This is consistent with our view that the return was more or less what would be expected in a low-productivity regime. It is interesting to note that the average survival of all stocks was low for the 1958 brood year, including the Chilko smolt data series. The uniformity of this response indicates that ocean survival is the most probable reason for the poor returns. Extreme data points are valuable in any important data series and because they are extreme, they are both rare and frequently controversial. The 1958 data point is important in this study because it represents the only year of large escapements in the early regime relative to the larger escapements in the recent regime.

The climate event of 1976–1977 is not as well known as the El Niño events even though the 1976–1977 event represented a long-term shift, and the El Niño events tend to represent short-term variability. There was not a consistent relationship between El Niño events and sockeye production, indicating that the shift in production was in response to a longer term signal. The existence of long-term signals in trends of plankton abundance and the relative absence of El Niño signals have recently been described for plankton off the west coast of Vancouver Island (Mackas 1995). Thus, while there may be specific El Niño events that affect production, our examination of the relationship between El Niño events and anomalous brood year production did not identify a consistent response of Fraser River sockeye stocks.

For all four cycles it is clear that there was a synchronous response between the Fraser River returns and the all-nation minus Fraser River catch of sockeye for the later regime. Much of this increase in catch occurred outside of Canada, particularly in Alaska (Hare and Francis 1995), but the increases in Canada were large relative to the earlier regime. In 1993, sockeye salmon returns to the Fraser River were the largest in this time series, as they were throughout the North Pacific. Since 1993, returns to the Fraser River have been below expectations,

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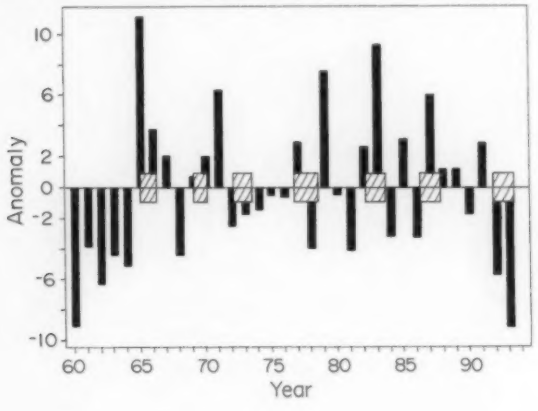
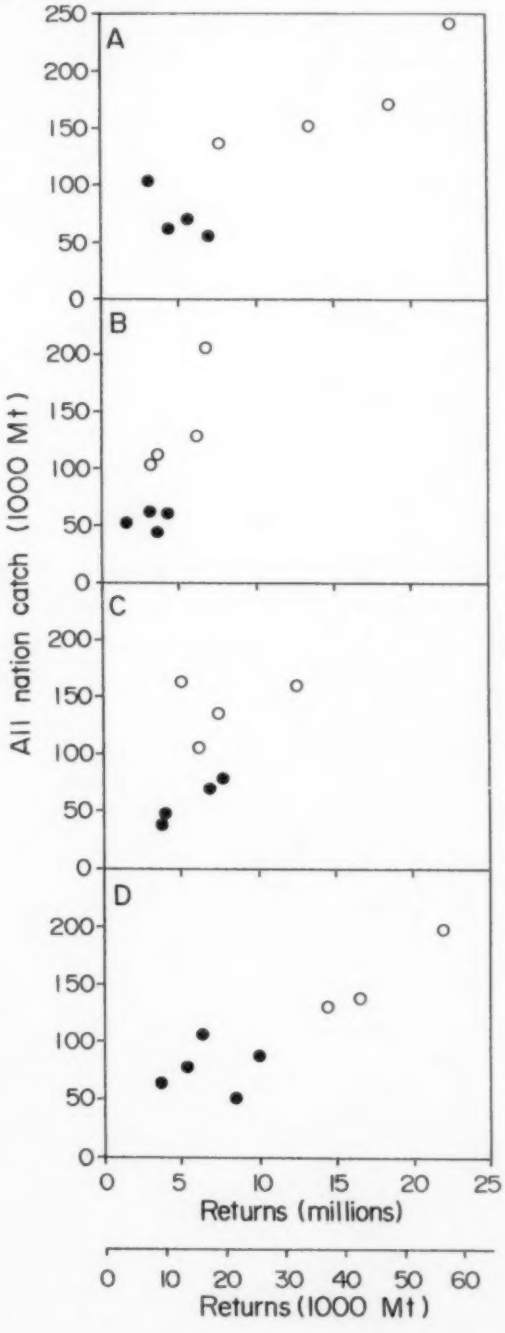
Fig. 11. The return for each spawning female, expressed as a yearly anomaly for the time series and identified as the year smolts went to sea. A higher return per female produces a more positive anomaly. Hatched areas represent the El Niño events.



while returns to Alaska have remained high, possibly indicating a change in the synchrony among stocks. Thus, we will have to wait to find out what happens to both Alaskan production and Fraser River sockeye production. Past relationships for Fraser River stocks may not necessarily persist into the future, particularly if future changes in climate affect sockeye salmon more at the southern limits than their northern limits in a manner analogous to the impacts on coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) off Oregon compared with areas farther north (Pearcy 1992).

Even though the Fraser River sockeye salmon data are among the best available, these data, like less-studied fish stocks, have large and unexplained interannual variability in production estimates. Studies of climate impacts on salmon population dynamics are also constrained by our poor understanding of the mechanisms involved. It is possible that, in the unfished state or with only low harvest rates, the impacts of climate would be clearer. However, the differences in productivity before and after the 1976–1977 climate shift were sufficiently clear that we concluded that fish populations can be affected by large-scale changes in climate and that the impacts may appear as rather abrupt shifts in the trends in productivity. Accepting this, there are some obvious implications for fisheries management. There is a need to be able to distinguish the impacts of fishing from the impacts of a changing environment. In particular, a shift to a lower or higher productivity regime is an important reference point for fisheries managers and needs to be detected. In fact, when time series are used in fisheries assessments, there should be a commentary on the validity of assuming that the data are one continuous series. Concern over the incorporation of production relationships into stock assessments using time series that may be confounded by the impacts of climate change is not new (Walters 1987). Natural, decadal shifts in productivity would also have to be identified and understood to be able to interpret fishing effects. The impacts also have important economic implications as management may not always be equated to producing more fish. For example, increasing escapements or artificial

Fig. 12. The total returns to the Fraser River compared with the total all-nation catch minus Fraser River catch for the same year, separated by the four cycles of production. The solid circles are the years from 1960 to 1976 and the open circles are from 1977 to 1990: (A) 1990 cycle, (B) 1987 cycle, (C) 1988 cycle, and (D) 1989 cycle. The additional *X* axis provides a comparison of Fraser River sockeye production with all-nation catch, which is recorded in metric tons.



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enhancement may be an appropriate strategy during a productive regime; however, rebuilding sockeye salmon stocks or other stocks to historically high levels of abundance may not be possible in the low-productivity regimes.

The concept of changing, decadal-scale trends in fish populations suggests that the stewardship of our marine ecosystems will require a better understanding of the mechanisms that affect the abundance of fishes. Certainly, we need to understand more about the concept of natural regime shifts if we are to be able to detect global warming impacts, which may also appear as shifts in productivity. Steele (1996) viewed the past problems in fisheries management as the statistical uncertainty associated with estimating cohort strength. He believes the next generation of problems will be the conceptual uncertainty about recruitment at decadal time scales. We think that a better understanding of these natural shifts in production trends is the kind of new information that will contribute to the improvement of fisheries management as discussed by Walters and Parma (1996).

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Intraspecific genetic structure of white sucker (*Catostomus commersoni*) in northeastern North America as revealed by mitochondrial DNA polymorphism

Pascale Lafontaine and Julian J. Dodson

Abstract: Restriction fragment length polymorphisms in mitochondrial DNA (mtDNA) were used to study the influence of Pleistocene glaciations on the intraspecific genetic structure and distribution of the white sucker (*Catostomus commersoni*) in northeastern North America. A total of 312 white sucker from 13 populations, including a population of dwarf ecotypes (*Catostomus commersoni utawana*), were analysed. An average of 93 fragments per individual and 40 haplotypes were generated by nine restriction endonucleases. Four discrete clades were identified but the majority of the genotypes found (70%) were not associated with any of the clades. The phylogenetic continuity and the geographic admixture of some of the few clades identified are associated with the extensive distribution of the species south of the ice sheets during Pleistocene glaciation events. Hierarchical analysis of the variability in mtDNA revealed a significant regional subdivision to the northwest and southeast of the St. Lawrence drainage system and significant structure at the population level. As the dwarf form exhibited a haplotype identical to that found among normal forms, we tentatively conclude sympatric divergence as the most likely origin of the dwarf and normal ecotypes sampled in the Adirondacks.

Résumé : Nous avons utilisé les polymorphismes de longueur de fragment de restriction sur l'ADN mitochondrial (ADNmt) afin d'étudier l'influence des glaciations du Pleistocène sur la structure génétique intraspécifique et sur la distribution du meunier noir (*Catostomus commersoni*) au nord-est de l'Amérique du Nord. Un total de 312 meuniers noirs provenant de 13 populations, dont une comprenant un écotype nain (*Catostomus commersoni utawana*), ont été analysés. Une moyenne de 93 fragments par individu et 40 haplotypes ont été générés par neuf endonucléases de restriction. Quatre petits groupes distincts ont été identifiés mais la majorité des génétypes retrouvés (70%) n'ont été associés à aucun de ces groupes. La continuité phylogénétique et le chevauchement de la distribution de quelques un de ces groupes identifiés sont associés à la large distribution de l'espèce au sud de la calotte de glace lors des événements glaciaires du Pléistocène. L'analyse hiérarchique de la variabilité de l'ADNmt révèle une subdivision régionale significative entre le nord-ouest et le sud-est du bassin de drainage du fleuve St-Laurent ainsi qu'une structure significative au niveau de la population. Comme la forme naine présentait un haplotype identique à celui retrouvé parmi la forme normale, nous suggérons une divergence sympatrique comme explication la plus probable de l'origine de l'écotype nain et normal échantilloné dans les Adirondacks.

Introduction

Historical biogeographical events have played an important role in shaping contemporary intraspecific genetic structure (Bermingham and Avise 1986). Geological and other historical events have changed the distributional areas of organisms, built up or suppressed geographic barriers, and gathered or isolated genetic populations from the rest of the species. The Pleistocene glaciations caused important changes in the distribution and genetic structure of North American fishes. Glaciation is believed to have decreased genetic diversity through population-bottleneck effects and to have confined populations of northern temperate fishes in separate glacial refugia where they evolved independently for thousands of years, resulting in the development of genetically distinct intraspecific groups (Bernatchez 1995; McAllister et al. 1986). When retreating, the ice cover left huge postglacial lake and river connections that provided formidable opportunities for the dispersal and admixture of these previously isolated groups (Bernatchez and Dodson 1991).

Studies of restriction fragment length polymorphisms (RFLPs) in the mitochondrial DNA (mtDNA) of northern fishes have clearly illustrated the impact of glaciation on intraspecific genetic structure. Haploid, maternally inherited, and devoid of crossing over, mtDNA is a powerful instrument to detect genetic variation within and between fish populations. The ability of mtDNA to retain a history of past isolation, in spite of contemporary admixture, is well demonstrated in a variety of species (Bermingham and Avise 1986; Avise et al. 1987; Billington and Hebert 1991; Avise 1992). Among northern fishes, mtDNA analyses of walleye (*Stizostedion vitreum*) (Billington et al. 1992), lake trout (*Salvelinus namaycush*) (Grewe and Hebert 1988), whitefish (*Coregonus clupeaformis*) (Bernatchez and Dodson 1991), cisco (*Coregonus artedii*) (Bernatchez and Dodson 1990*a*), and rainbow smelt (*Osmerus mordax*) (Bernatchez 1995) have all revealed the presence of major, intraspecific genetic discontinuities related to the

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Fig. 1. Maximum extent of the Wisconsin glaciation (solid line), distribution of white sucker (stippled area), and location of the principal glacial refugia: 1, Beringian; 2, Columbian; 3, Banff–Jasper; 4, Missourian; 5, Mississipian; 6, Atlantic.

genetic divergence of intraspecific groups of fish occupying different glacial refugia at various times during the Pleistocene.

White sucker, *Catostomus commersoni*, is one of the most abundant and widely distributed fish species in North America (Scott and Crossman 1974). Much of the present distributional area of white sucker was covered by the ice cap during the Pleistocene, except for the southern part, which remained ice free (Fig. 1). During the last glaciation, white sucker probably survived in refugia located south of the ice sheets that may have favoured the evolution of intraspecific genetic groups in allopatry. Thus, recolonization of the newly deglaciated areas of North America may have involved several distinct intraspecific genetic groups. White sucker found in the northeastern part of North America may have originated from one or all of three glacial refugia: the Mississippi, Missouri, and Atlantic (Crossman and McAllister 1986). Hudson Bay drainages may have been recolonized from the Mississippi and (or) the Atlantic glacial refugium. White sucker from the Great Lakes may have come from the Mississippi and (or) from the Missouri glacial refugium. The St. Lawrence valley may have been invaded by fishes coming from the Mississippi and the Atlantic glacial refugia (Underhill 1986). Although only one genetic group of white sucker would be expected along the east coast of North America, Schmidt (1986) proposed that, on the basis of the wide distribution of the species, it may be possible to find more than one genetic group in the Atlantic region. All of these scenarios remain speculative. Therefore, the major objective of this study was to document the influence of glaciation on the intraspecific genetic structure of white sucker by relating intraspecific genetic divergence among contemporary populations to their distribution in northeastern North America.

We also investigated a special ecotype of the white sucker: a dwarf form (*Catostomus commersoni utawana*) lives in sympatry with the normal ecotype in the Adirondack Mountains (New York state, U.S.A.). Dwarf white sucker apparently live deep in lakes; the spawning period is a few weeks later than the spawning period of normal white sucker and the length at maturity ranges between 127 and 203 mm, compared with 310 and 460 for the normal ecotype. Dwarf white sucker grow slowly but mature 1 year before the normal ecotype, which matures at 4 years of age in this area (Dence 1948; Beamish 1970).

Surprisingly widespread, dwarfism is found among many fish species, especially salmonids. Studies have shown that two mtDNA scenarios are associated with this phenomenon. In the case of whitefish, Bernatchez and Dodson (1990*b*) demonstrated that the sympatric occurrence of the dwarf and normal ecotypes in the Allagash basin, Maine, U.S.A, resulted from a secondary contact between two phylogenetic groups of allopatric origin. The same scenario was presented to explain the origin of different ecotypes of whitefish in the Squanga Creek drainage, Yukon (Bernatchez et al. 1996).

Dwarfism is also present among limnetic smelt, *Osmerus mordax*, but Taylor and Bentzen (1993) presented evidence that sympatric divergence was responsible for the presence of the two ecotypes. In fact, dwarf and normal smelt belong to the same mtDNA genetic clade and must have diverged from a common ancestor. Their morphological differences are characters important in foraging: dwarf smelt have more gill rakers and larger eyes, but shorter upper jaws and shallower heads than normal and anadromous smelt. According to these morphological traits, dwarf smelt are planktivorous whereas normal and anadromous smelt are macrophagous and commonly piscivorous (Taylor and Bentzen 1993). Therefore, the second objective of this study was to determine the phylogenetic relationship of dwarf and normal white sucker. The occurrence of phylogenetically distinct mtDNA haplotypes discriminating dwarf white suckers from normal ecotypes would be consistent with a scenario of allopatric divergence.

Materials and methods

Sampling

Between 2 and 68 fish were sampled from each of 13 sites situated in the northeastern part of the white sucker's distribution (Table 1 and Fig. 2). Dwarf white sucker were sampled in Wolf Lake, New York, where normal sucker are usually found in sympatry. Biologists from the Adirondack Ecological Center also sampled normal white sucker in Catlin Lake, adjacent to Wolf Lake, where dwarf sucker are also present. Other sites were sampled either by the authors or by collaborators. Fish were immediately dissected to remove liver and eggs, which were subsequently frozen or kept on wet ice prior to mtDNA extraction.

Mitochondrial DNA extraction and analysis

Mitochondrial DNA was successfully extracted and analysed for 312 fish. Tissues were extracted using the method of Bernatchez et al. (1988). Aliquots of mtDNA were digested separately with two hexameric (*Dra*I, *Xba*I), four multihexameric (*Acc*I, *Ava*I, *Ban*II, *Hin*cII), and three multipentameric (*Ava*II, *Ban*I, *Nci*I) restriction endonucleases. Mitochondrial DNA fragments were electrophoretically separated on 0.8, 1.0, or 1.2% agarose gels for 4 h at 75 V.

Ethidium bromide staining revealed restriction fragments when mtDNA was obtained in sufficient quality and quantity. In cases of low or poor-quality yield, mtDNA fragments were transferred to nylon membranes under alkaline conditions and hybridized with a pure radiolabelled *Catostomus commersoni* mtDNA pool probe prepared

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Table 1. Sampling locations, sample sizes, and number of mtDNA haplotypes for populations of *Catostomus commersoni*.

	Sampling site	Sample size	Number of haplotypes
a	Lake Manitoba (Manitoba)	26	8
b	Jack Fish Bay (Lake Superior, Ont.)	24	8
c	Gatineau River (Quebec)	73	12
d	St-Maurice River (Quebec)	68	12
e	Sans nom River (Mastigouche, Que.)	17	1
f	Great Whale River (North Quebec)	2	2
g	St. Lawrence River (Quebec City)	18	8
h	Yoyo Lake (Forestville, Que.)	22	1
i	Amédée River (Baie-Comeau, Que.)	3	3
j	Cliff Lake (Maine)	13	2
k	Catlin Lake (New York state)	15	4
l	Wolf Lake (New York state)	12	3
m	St. Andrews (New Brunswick)	19	1

Note: Letters (a–m) correspond to sampling sites illustrated in Fig. 2.

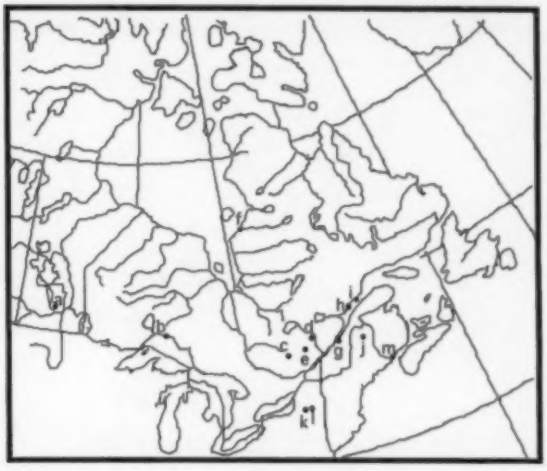
with the multiprimed (Amersham) DNA labelling reaction. Membranes were submitted to stringent washings at 68°C and were then autoradiographed using an intensifying screen for 5–36 h at –70°C.

Sizes of fragments were estimated by running simultaneously into the agarose gel digests of phage lambda DNA with *Hin*dIII and *Eco*RI–*Hin*dIII double digest. Some fragments less than 300 base pairs (bp) in length could not be scored. Distinct single endonuclease patterns were identified by a specific letter. Each fish was assigned a multiletter code that described its composite mtDNA genotype.

Restriction-site differences between genotypes were inferred from changes in fragment patterns because such changes could be readily explained by specific site gains or losses. Estimates of nucleotide sequence divergence (p) among mtDNA haplotypes calculated by the site approach of Nei and Li (1979) were used to estimate genetic distance among haplotypes. The resulting distance matrix served to construct a phenogram using the unconstrained branch-length clustering method of Fitch and Margoliash (1967) (program FITCH in the PHYLIP 3.4 computer package, Felsenstein 1992). In addition, a site presence–absence matrix was used to generate phylogenetic trees according to Wagner parsimony criteria using the MIX program from PHYLIP. Symplesiomorphic and autapomorphic characters were omitted from the site matrix. Confidence in parsimony trees was assessed by bootstrap resampling of the restriction site presence–absence matrix (N = 10 000) using SEQBOOT of PHYLIP. A majority-rule consensus tree was built using CONSENSE of PHYLIP. Phylogenetic trees were rooted using a haplotype of longnose sucker (*Catostomus catostomus*), sampled in the St. Lawrence River (Quebec City). As a measure of inter- and intra-population genetic diversity, we computed the maximum likelihood estimation of the average number of nucleotide substitutions per site within populations (nucleotide diversity) and between populations (nucleotide divergence) (Nei and Tajima 1981, 1983; Nei 1987). The neighbour-joining program of PHYLIP was used to cluster populations according to nucleotide divergence.

We estimated the likelihood of detecting mitochondrial DNA diversity at the population level using the combinatorial approach of Hebert et al. (1988) as applied by Bernatchez et al. (1989) to mtDNA data. The relationship between the number of fish sampled and the number of mtDNA genotypes detected was estimated for each population by an incremental random choice of individuals (increments of 4 fish to a maximum of 312 or 204 fish). The procedure was repeated 10 times for each sampling intensity to generate an estimate of random sampling variance. This procedure was done on all of the 312 fish of the sampled area and also on the 204 fish from the St. Lawrence drainage system. The relationship between the number of genotypes

Fig. 2. Location map of *Catostomus commersoni* sampling sites named in Table 1.



detected as a function of genome sampling was estimated for each population by incremental random choice of restriction enzymes. Results were obtained by an incremental choice of one enzyme repeated 10 times for each incremental step. As different restriction enzymes produced different numbers of fragments, we attributed the fragment numbers to their respective enzymes and summed the fragments for any enzyme combination.

Geographical heterogeneity in the frequency of mtDNA RFLP haplotypes among sampling sites in northeastern north America was analyzed using analysis of molecular variance (AMOVA) (Excoffier et al. 1992). We set interhaplotypic distances as equal for the analysis of molecular variance. Two geographical groups were formed with the sampled populations: northwest of the St. Lawrence River (Lake Manitoba, Jack Fish Bay, Gatineau River, St. Maurice River, Sans nom River, Great Whale River, Yoyo Lake, and Amedée River) and southeast of the St. Lawrence River (Cliff Lake, Catlin Lake, Wolf Lake, and St. Andrews). The St. Lawrence population was eliminated because of its central position in this distribution. F_{st} and associated p values ($p < 0.05$) were calculated within and between the two geographical groups.

Results

The nine endonucleases generated a total of 191 restriction fragments with a mean of 93 fragments per individual. Seven out of nine enzymes were polymorphic and discriminated a total of 40 mtDNA haplotypes among the 312 fish analysed from 13 sampling sites (Tables 1 and 2; Fig. 2). Fragment patterns generated by each enzyme and restriction-site differences between genotypes inferred from changes in fragment patterns are presented in Figs. 3 and 4, respectively. The 150 restriction sites so identified included 59 synapomorphic sites. The mean size of the mtDNA genome, estimated by averaging the sum of all digestion patterns, was 17 769 bp (SD = 1295), similar to the length estimates obtained for a variety of other fish species (Billington and Hebert 1991). The mean pairwise sequence divergence was 0.70 ± 0.34% and ranged from 0.1 to 2.4% among all white sucker lineages.

An analysis of sequence divergence between haplotypes of white sucker using the site method and clustered with Fitch revealed no major divisions in the tree (Fig. 5). Small clusters

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Table 2. Definitions of composite haplotypes, absolute frequency (*n*), and distribution of the mtDNA haplotypes resolved among *Catostomus commersoni*.

Composite haplotypes										*n*	Locality
1	A	A	A	A	A	A	A	A	A	3	c
2	A	A	A	A	A	A	A	A	D	1	i
3	A	A	A	A	A	A	A	B	D	1	g
4	A	A	A	A	A	A	B	A	E	2	d
5	A	A	A	A	A	A	B	A	H	1	k
6	A	A	A	A	A	A	B	B	E	34	g, j, m
7	A	A	A	A	A	A	C	B	E	1	j
8	A	A	A	A	A	B	A	A	A	73	c, d, e
9	A	B	A	A	A	B	A	A	A	1	c
10	A	A	A	A	A	B	A	A	D	1	d
11	A	A	A	A	A	B	B	A	A	1	c
12	A	A	A	A	B	A	A	A	A	1	c
13	A	A	A	A	B	A	A	A	B	13	b, c, d
14	A	A	A	A	B	A	A	A	C	5	c, d, g, i
15	A	A	A	A	B	A	A	A	E	2	a, b
16	A	B	A	A	B	A	A	A	E	9	a
17	A	A	A	A	C	A	A	A	B	2	d, f
18	A	A	A	A	C	A	A	A	C	45	b, c, d, f, g
19	A	A	A	A	C	A	A	A	D	2	d, g
20	A	A	A	A	C	A	A	A	E	2	a
21	A	A	A	A	C	A	A	A	F	2	b, d
22	A	A	A	A	C	A	A	A	G	4	d
23	B	B	A	A	C	A	A	A	E	1	c
24	A	C	A	A	C	A	A	A	E	1	g
25	A	A	A	A	D	A	A	A	A	7	c
26	A	A	A	A	D	A	A	A	B	2	d
27	A	A	A	A	D	A	A	A	D	1	k
28	A	A	A	A	E	A	A	A	E	22	a, b
29	A	A	A	A	E	A	A	A	H	1	a
30	A	A	A	A	E	A	A	A	I	2	b
31	A	A	A	A	F	A	A	A	D	56	c, d, g, h, i, k, l
32	A	A	A	A	F	A	D	A	D	2	l
33	A	A	A	A	G	B	A	A	A	1	c
34	A	A	A	A	H	A	A	A	A	1	g
35	A	B	A	A	H	A	A	A	E	1	a
36	A	B	A	A	I	A	A	A	J	1	a
37	A	B	A	A	J	A	A	A	E	3	a, b
38	A	A	A	A	K	A	A	A	D	1	k
39	A	A	A	A	L	A	A	A	D	1	l
40	A	A	A	A	M	A	A	A	E	2	b

Note: Restriction enzymes are in order *Xba*I, *Dra*I, *Acc*I, *Ava*I, *Ava*II, *Ban*I, *Ban*II, *Hin*cII, *Nci*I. Capital letters identify fragment patterns presented in Fig. 3. Small letters refer to sample locations illustrated in Fig. 2.

of haplotypes were observed throughout the tree, but no discontinuities divided the tree into major subgroups.

The majority-rule consensus tree also failed to reveal significant division of the species into major clades. Rather, four small clades, also visible in the Fitch phenogram, were resolved (Fig. 6). These clades constituted only 30% of the haplotypes. Seventy percent of the genotypes were poorly resolved and could not be associated with a distinct clade. Haplotype 36, from Lake Manitoba, was a highly divergent haplotype distinct from all others.

Parsimony analysis of the *Ava*II and *Nci*I restriction fragment patterns revealed sites that were hypervariable. Such homoplasy could mask some important groupings among genotypes by reducing the resolution of the phylogenetic trees. However, removing these sites from the input matrix and producing a new majority-rule consensus tree did not alter tree topology. Thus, we concluded that the absence of major discontinuities in the tree was not due to homoplasy.

Group A

This clade comprised two haplotypes: No. 13 found in Jack Fish Bay, the Gatineau River, and the St. Maurice River and No. 17 from the St. Maurice River and Great Whale River (Tables 2 and 3). The clade was supported at the 58% level in the majority-rule consensus tree. It was restricted to the west of the St. Lawrence – Great Lakes axis (Fig. 7). The pairwise sequence divergence within this group was 0.2%. Restriction patterns B and C of *Ava*II and B of *Nci*I were diagnostic for this group (Table 2).

Group B

This clade comprised two haplotypes: No. 31 from the Gatineau River, St. Maurice River, St. Lawrence River, Yoyo Lake, Amédée River, Catlin Lake, and Wolf Lake and No. 32 from Wolf Lake (Tables 2 and 3). It was supported at the 82% level in the majority-rule consensus tree. This clade was principally located to the east of the St. Lawrence River (Fig. 7). *Ava*II restriction pattern F was diagnostic for the group (Table 2). The pairwise sequence divergence within this group was 0.2%. The clade comprised the majority (11 out of 12 fish) of the dwarf white suckers (*Catostomus commersoni utawana*) sampled in Wolf Lake (Table 1).

Group C

This clade comprised five haplotypes: No. 4 from the St. Maurice River, No. 5 from Catlin Lake, No. 6 from the St. Lawrence River, Cliff Lake, and St. Andrews, No. 7 from Cliff Lake, and No. 11 from the Gatineau River (Tables 2 and 3). This was supported at the 53% level in the majority-rule consensus tree. Clade C showed the most easterly distribution of the clades resolved in this study (Fig. 7). The mean pairwise sequence divergence within this group was 0.38 ± 0.25%. *Ban*II restriction fragments B and C were diagnostic for the group (Table 2).

Group D

This group comprised three haplotypes: Nos. 28 and 29 from Lake Manitoba and Jack Fish Bay and No. 30 from Jack Fish Bay (Tables 2 and 3). This was supported at the 67% level in the majority-rule consensus tree. This clade presented the most westerly distribution among sampled haplotypes (Fig. 7). The mean pairwise sequence divergence within this group was 0.13 ± 0.06%. *Ava*II fragment pattern E was diagnostic for this group (Table 2).

The maximum likelihood estimation of the average number of nucleotide substitutions per site within populations (nucleotide diversity) and between populations (nucleotide divergence) did not clearly reveal population groupings. The population sampled near Quebec City was separated from all of the others by 0.16% sequence divergence (Fig. 8). This population also presented the highest nucleotide diversity (0.57 ± 0.06%).

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Fig. 3. Fragment size estimates (in base pairs) of all fragment patterns observed among white sucker and longnose sucker (Z). Asterisks identify fragments that were not observed but were assumed under the criterion of the minimum mutational steps involved in fragment changes. The presentation of two 1825-bp fragments for *Ban*II haplotype D indicates that two superimposed 1825-bp fragments were observed. The presentation of two or more fragments of the same size for different haplotypes of the same restriction enzyme indicates different origins of similar-sized fragments.

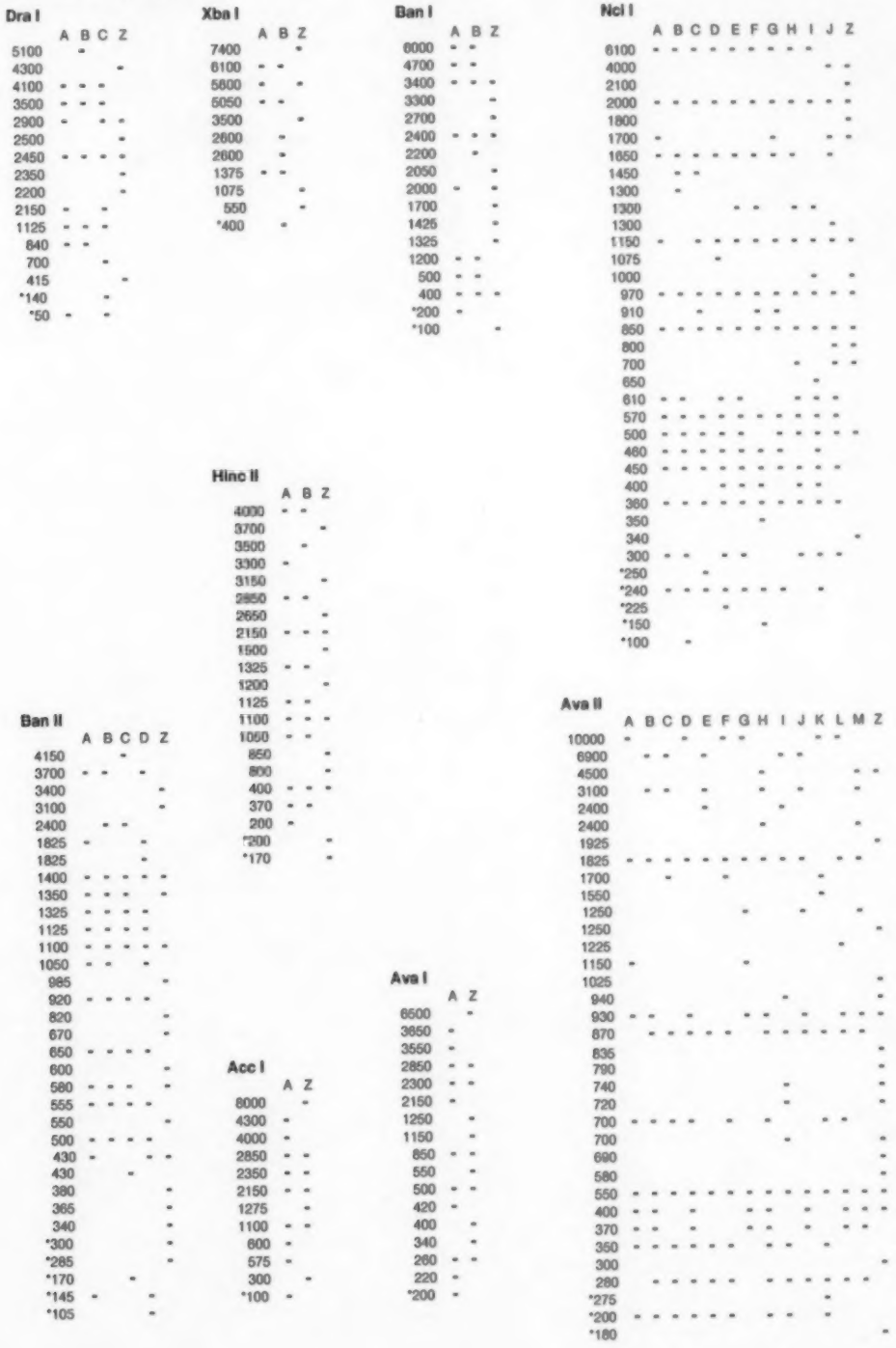

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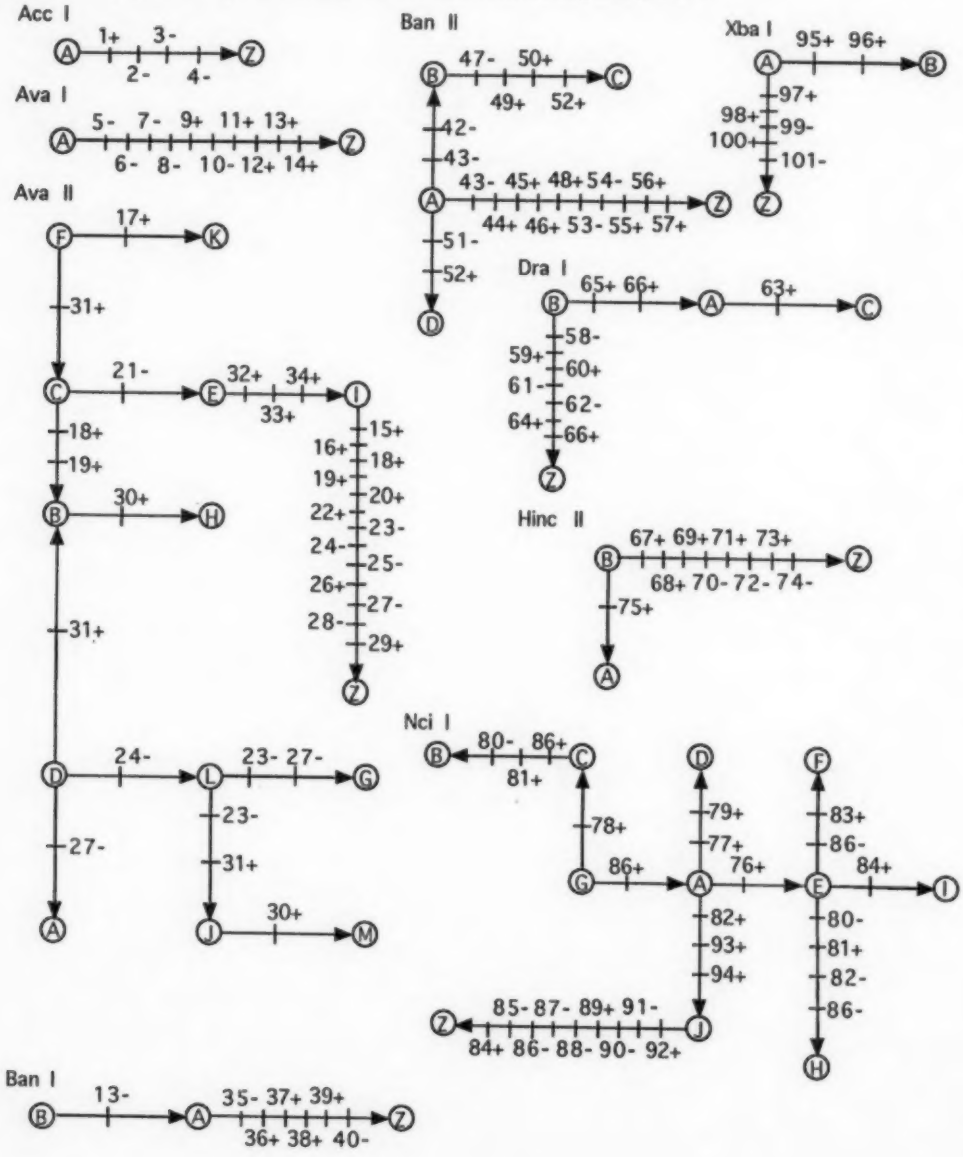
Fig. 4. Parsimonious, unrooted networks illustrating relationships among fragment patterns observed for all restriction enzymes described in Fig. 3. Site changes (losses (–) or additions (+)) involved in moving between fragment patterns (letters) are numbered along branches (arrows indicate direction of changes) and were parsimoniously deduced from observed fragment patterns.



Hierarchical analysis of the total variation in mtDNA of white sucker revealed a significant regional subdivision in eastern North America and significant structure at the population level. Divergence between groups sampled to the northwest and southeast of the St. Lawrence River was significant and accounted for 14.30% of the variation ($p < 0.031$). Populations within regions were significantly differentiated and accounted for 35.63% of the variation ($p < 0.001$). The largest fraction of total variation was found within populations (50.07%, $p < 0.001$).

The number of mtDNA genotypes detected as a function of the sample size of specimens and restriction fragments increased rapidly for the whole area studied (Fig. 9). A sample size of more than 312 specimens and 96 restriction fragments was inadequate to detect all mtDNA variants within the sampled area (Figs. 9, 10). In the case of the St. Lawrence drainage, sample sizes of up to 204 specimens were not sufficient to detect all mtDNA variants (Fig. 11).

Discussion

The mean pairwise intraspecific sequence divergence of

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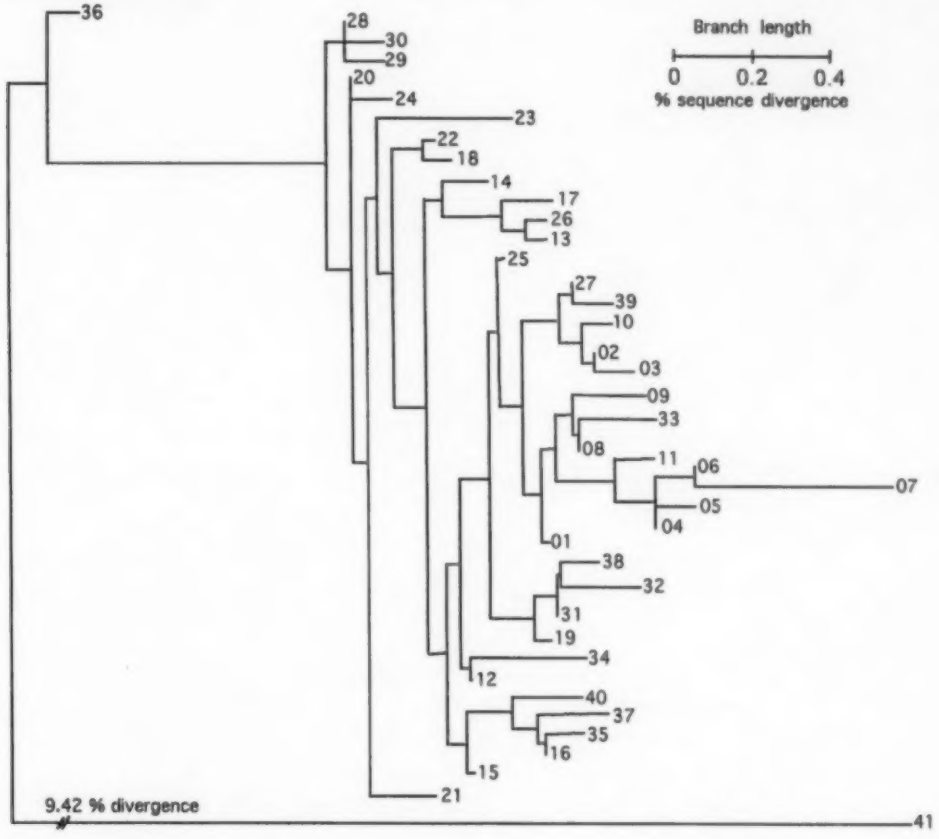
Fig. 5. Fitch phenogram clustering the distance matrix, on the basis of restriction sites, of percent sequence divergence among 40 mtDNA haplotypes described for *Catostomus commersoni*. Numbers refer to haplotypes defined in Table 2. The tree was rooted with haplotype 41 represented by *Catostomus catostomus*.



0.70 ± 0.34%, ranging from 0.1 to 2.4%, is the greatest observed to date among northern fishes: *Coregonus clupeaformis* (0.36 ± 0.16%; range 0.07–0.86%), *Stizostedion vitreum* (0.47 ± 0.20%; range 0.15–0.60%), *Coregonus artedii* (0.52 ± 0.22%; range 0.08–1.03%), and *Salvelinus fontinalis* (0.62 ± 0.34%; range 0.11–1.32%). These four species and *Salvelinus namaycush* found in northeastern North America are composed of two or three genetic clades separated by many mutational steps and display heterogeneous geographic distributions (Grewe and Hebert 1988; Billington and Hebert 1988; Bernatchez and Dodson 1990*a*, 1990*b*; Bernatchez and Danzmann 1993). The most commonly accepted explanation for major genetic discontinuities with heterogeneous geographic distributions involves long-term barriers to gene flow, such that conspecific populations occupy easily recognizable branches on an intraspecific phylogenetic tree (Avise et al. 1987). In northern North America, such long-term barriers to gene flow are associated with the refugia created during Pleistocene glaciation events. Genetic groups with more easterly distributions are generally attributed to any number of Atlantic refugia (eastern coastal plains and exundated banks), whereas those with a more westerly distribution are attributed to the Mississippi or Missouri refugium. These genetic groups are deduced from phenograms and cladograms exhibiting genetic breaks or discontinuities that form clusters of haplotypes.

In the present study, a major genetic discontinuity was observed between haplotype 36 and all other haplotypes in Fitch and the majority-rule consensus tree. However, within the majority cluster, no major discontinuities were observed. Rather, four small clades separated by few mutational steps from other haplotypes were defined. Haplotypes of clade D are clearly associated with a westerly distribution, being found only in Manitoba and Lake Superior. Clade C is mostly located in the Atlantic region, but some haplotypes (Nos. 4, 5, 11) are found in more central regions. Clades A and B exhibit a west–east distribution relative to the St. Lawrence system, with some intermingling to the west of the St. Lawrence (Table 3). Most haplotypes (70%) were poorly resolved by the majority-rule consensus tree. The majority of fish characterized by these haplotypes were found west of the St. Lawrence River. Although a neighbour-joining phenogram showed no significant population groupings, an AMOVA based uniquely on the frequency distributions of haplotypes revealed that dividing the distribution of white suckers to the east and west of the St. Lawrence River accounted for a significant portion of the total

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Fig. 6. Majority-rule consensus tree clustering 40 mtDNA haplotypes of *Catostomus commersoni* and their geographical distribution. Numbers at forks indicate the percentage of time the group consisting of the haplotypes located to the right of the fork occurred among the trees, out of 10 000 trees. The tree was rooted with *Catostomus catostomus*, haplotype 41. Numbers at the end of the tree forks indicate haplotypes defined in Table 2. Letters (a–m) refer to sampling sites identified in Table 1 and numbers below sampling sites are absolute abundances. The principal mtDNA clades are identified with vertical lines.

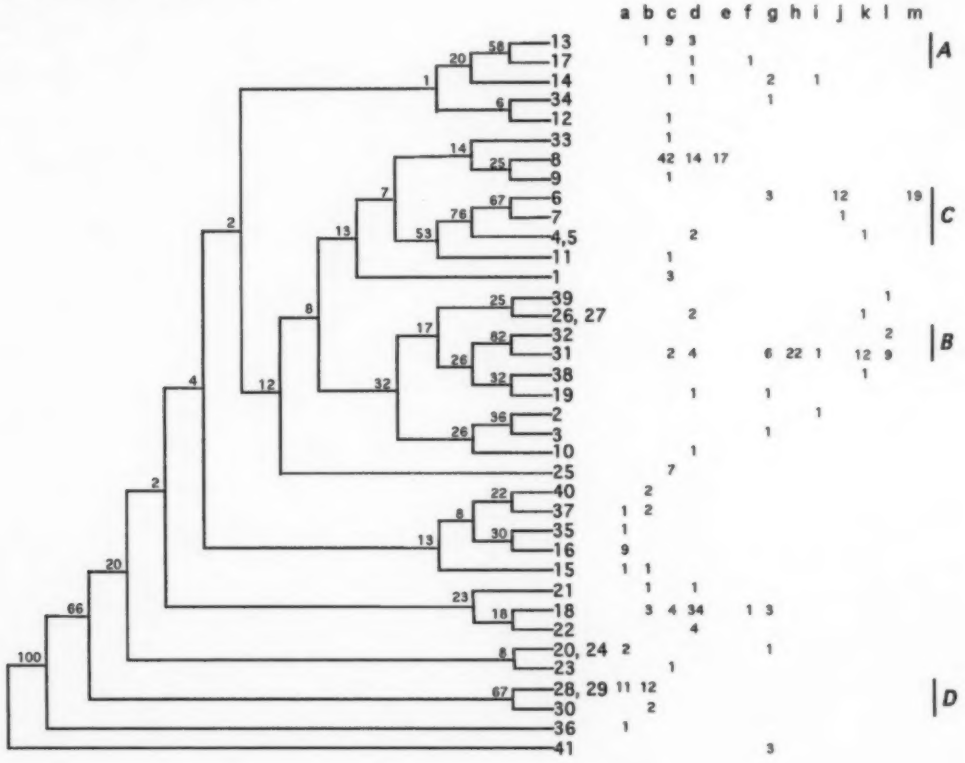
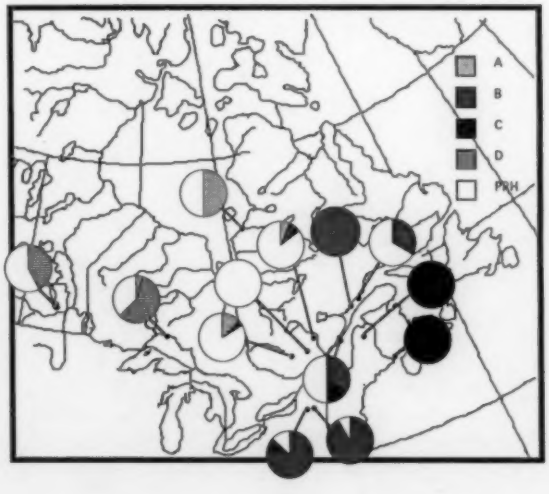


Table 3. Raw data for the geographic distribution of fish classified according to the four mtDNA clades and the poorly resolved haplotypes (PRH).

Clade/site	a	b	c	d	e	f	g	h	i	j	k	l	m
A	0	1	9	4	0	1	0	0	0	0	0	0	0
B	0	0	2	4	0	0	6	22	1	0	12	11	0
C	0	0	1	2	0	0	3	0	0	13	1	0	19
D	11	14	0	0	0	0	0	0	0	0	0	0	0
PRH	15	9	61	58	17	1	9	0	2	0	2	1	0

Note: Capital letters refer to genetic groups described in Fig. 7 and lowercase letters refer to sampling sites described in Fig. 2.

Fig. 7. The geographic distribution of the four discrete mtDNA clades. Letters refer to genetic groups described in Fig. 6. Poorly resolved haplotypes (PRH) are pooled in the same category. See raw data in Table 3.



genetic variation. The St. Lawrence sample stands apart, largely owing to high genetic diversity (Figs. 8, 11).

The existence of many closely related haplotypes that are only partially geographically localized has been associated with species or subsets of species with historically intermediate levels of gene flow between geographic populations (phylogeographic category V, Avise et al. 1987). In this scenario, ancestral haplotypes may be dispersed over a wide area whereas more recent mutations are confined to specific areas (e.g., *Amia calva*, Bermingham and Avise 1986). This explanation does not apply to the present situation. There is no doubt

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Fig. 8. Neighbour-joining phenogram clustering populations according to the distance matrix resulting from maximum likelihood estimation of the net average number of nucleotide substitutions per site between populations (nucleotide divergence). Intrapopulation nucleotide diversity is presented to the left of each population. The tree was rooted with the population of the St. Lawrence River (Quebec). Letters are populations identified in Table 1.

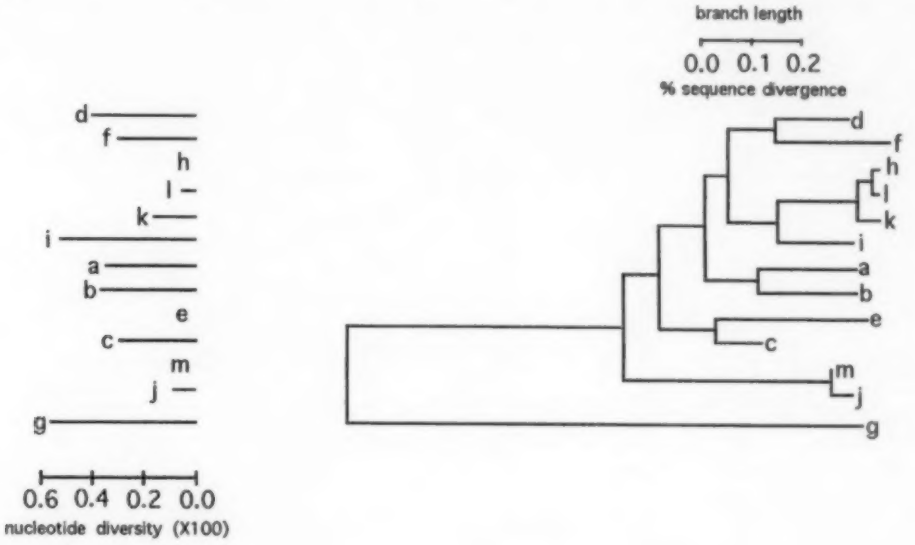


Fig. 9. Effect of fish sample size on the number of mtDNA clonal lines detected within all 13 populations of *Catostomus commersoni*. Incremental steps were stopped at 312 fish. Vertical bars are standard deviations based on 10 repetitions at each incremental step.

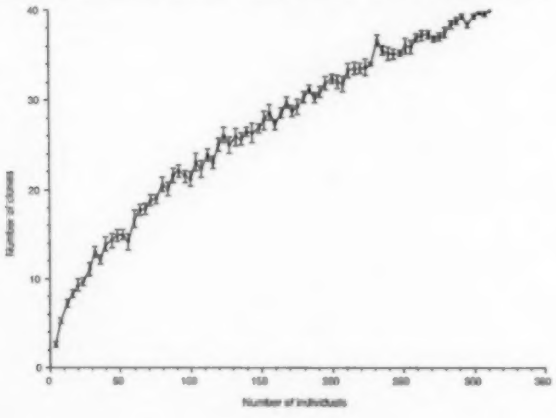
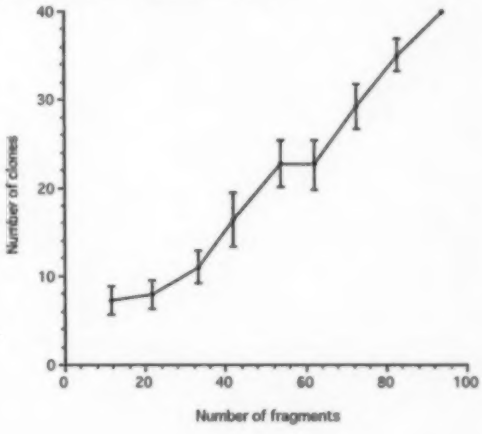


Fig. 10. Effect of number of restriction fragments assayed on the number of mtDNA clonal lines detected within all populations of white sucker studied. Vertical bars are standard deviations based on 10 repetitions at each incremental step.



that white sucker, over a large part of their present distribution, were confined to glacial refugia during Pleistocene glacial events that limited gene flow among populations. However, it is probable that white sucker were also extensively distributed to the south of the ice cap. The present distribution of the species includes areas that were well south of the maximum extent of the Laurentide ice sheet (Fig. 1). Thus, the northern part of the range may have been recolonized from multiple origins, contributing to the phylogenetic continuity of the species observed today. In addition, high genetic diversity may have been retained throughout the glacial distribution of the species that minimized the development of genetic discontinuities by stochastic lineage extinction. Avise et al. (1984) demonstrated that contemporary intraspecific mtDNA variability is influenced by stochastic lineage extinction, which in turn is a function of long-term effective population size. Today, the species is widely distributed and so abundant that it may represent more than half of the total fish biomass in some lakes (Chen and Harvey 1994). Thus, large effective population sizes in the past may have acted to buffer stochastic lineage extinction among geographically separated populations.

The four clades resolved in the majority-rule consensus tree involve haplotypes that diverged long before the last glaciation.

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Fig. 11. Effect of fish sample size on the number of mtDNA clonal lines of white sucker detected within populations of St. Lawrence drainage: Gatineau River, St. Maurice River, Sans nom River, St. Lawrence River (Quebec City), Yoyo Lake, and Amédée River. Incremental steps were stopped at 204 fish. Vertical bars are standard deviations based on 10 repetitions at each incremental step.

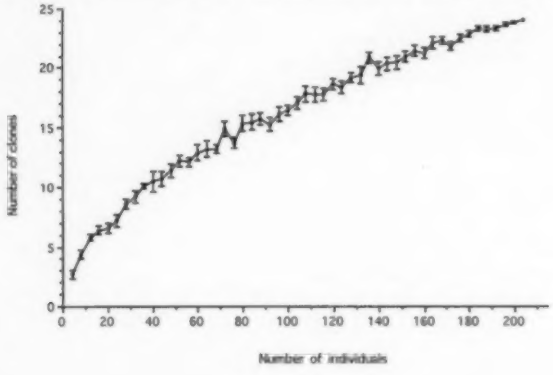
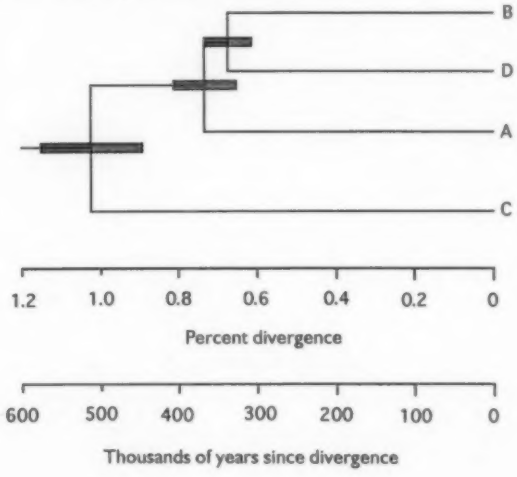


Fig. 12. Phenogram showing estimated percent sequence divergence among mtDNA of the four clades studied. Estimation of time of divergence is based on Brown's calibration of mtDNA evolution determined for mammalian species (Brown 1983). Horizontal bars represent standard deviations.



Assuming that the rate of divergence of 2% per million years calculated for mammals (Brown 1983) is a maximum rate for fish, clade C diverged from the others at least 500 000 years ago. The other clades diverged approximately 350 000 years ago (Fig. 12). However, the mitochondrial molecular clock was estimated at only 1% divergence per million years for three species of Pacific salmon (*Oncorhynchus* species) when molecular divergence estimates were calibrated with fossil evidence (Smith 1992). If this rate was applied to the present case, estimated divergence times would be doubled. In either case, the phylogenetic structure described here involves haplotype groupings that evolved during previous Pleistocene glaciations.

The dwarf white sucker population of Wolf Lake belongs to Clade B along with most other normal-sized white sucker of nearby Catlin Lake. One haplotype (No. 31) was the most common genotype characterizing both dwarf and normal ecotypes from the Adirondack sampling sites. It thus appears likely that both ecotypes were derived recently from a common ancestor. This scenario is comparable to the case of *Osmerus mordax* (Baby et al.1991; Taylor and Bentzen 1993) rather then the case of *Coregonus clupeaformis* (Bernatchez and Dodson 1991; Bernatchez et al. 1996). Unlike this latter study, however, the absence of other sampled lakes with dwarf sucker makes it impossible to assess the validity of a polyphyletic origin of dwarfism in this species. More intensive sampling in this region should be pursued to achieve this goal. It would also be important to find other regions where ecotypes are living in sympatry to determine if the divergence is truly sympatric or allopatric.

The attempt to relate haplotype groupings to recent glacial refugia is obviously hampered by the weak phylogeographic structure of the species. Clade D is most clearly related to a western distribution and may indicate recolonization from the Mississippi or Missouri refuge, as originally proposed by Crossman and McAllister (1986). The highly divergent haplotype No. 36 from Lake Manitoba suggests the existence of other divergent clades in the western part of their distribution, but this requires further sampling to confirm. Clade C appears to be Atlantic in origin, but it is present in the central part of the study region. The actual extent of this group will require a greater sampling effort. In fact, our sampling effort was not sufficient to document all of the genetic variability of the species in the study area (Fig. 9). Even with two thirds of all fish sampled coming from the St. Lawrence River drainage, sample size was not great enough to exhaust haplotype diversity (Fig. 11). Furthermore, addition of new restriction enzymes will surely reveal more polymorphic restriction patterns (Fig. 10). Nevertheless, available results illustrate that the St. Lawrence River delineates a transition zone in the recolonization of northeastern North America by white sucker, with fish of clades B and C largely restricted to areas located to the southeast of the St. Lawrence River. This suggests that these two clades were probably associated with Atlantic refugia located somewhere to the east of the Appalachians along the coastal plain of eastern North America.

In conclusion, the phylogeographic structure of the white sucker appears to be unique among fishes studied to date in northeastern North America. The lack of phylogenetic discontinuities and the intermingling of some of the few significant clades that do exist is in sharp contrast to other species sampled in this area. As this may be associated with an extensive distribution south of the ice sheet, a similar phylogeographic structure may be expected among other species with similar distributions and abundances.

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Effects of training on respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic swimming in juvenile rainbow trout (*Oncorhynchus mykiss*)

Randolph F. Lauff and Chris M. Wood

Abstract: Fuel utilization during aerobic exercise was determined in juvenile rainbow trout that had previously undergone 2 weeks of continuous aerobic training at 25% of their maximum sustainable speed (U_{crit}) and was compared with that in untrained trout. Instantaneous fuel usage was calculated from simultaneous measurements of oxygen consumption (M_{O_2}), carbon dioxide excretion (M_{CO_2}), and nitrogenous waste excretion (M_N). Over 58 h of sustained aerobic exercise at 55% of U_{crit}, M_{O_2} and M_{CO_2} remained virtually constant in the trained fish, in contrast to the significant fall over time in untrained fish, but total gas exchange was similar in the two groups. Aerobic respiratory quotient remained constant in the two treatments. M_N was lower in the trained fish, resulting in a much lower (and stable) nitrogen quotient; the nitrogen quotient increased with swimming duration in the untrained fish. Lipid was the major fuel source powering aerobic exercise in both groups. Carbohydrate also played a significant role, whereas protein was of lowest quantitative importance. Training resulted in a decrease in reliance on protein and an increase in reliance on lipid metabolism. The role of protein oxidation in supporting aerobic swimming in fish, which is smaller than commonly believed, is further reduced by training.

Résumé : Nous avons mesuré l'utilisation de l'énergie pendant l'exercice aérobie chez des truites arc-en-ciel juvéniles qui avaient été soumises à deux semaines d'entraînement aérobie continu à 25% de la vitesse soutenable maximale (U_{crit}), et nous avons comparé les résultats à ceux obtenus chez des truites non entraînées. L'utilisation instantanée de l'énergie a été calculée à partir de mesures simultanées de la consommation d'oxygène (M_{O_2}), de l'excrétion de dioxyde de carbone (M_{CO_2}) et de l'excrétion des déchets azotés (M_N). Sur une période de 58 h d'exercice aérobie soutenu à 55% de U_{crit}, M_{O_2} et M_{CO_2} sont demeurées pratiquement constantes chez les poissons entraînés, alors qu'elles ont nettement baissé avec le temps chez les poissons non entraînés, mais l'échange total de gaz était similaire chez les deux groupes. Le quotient respiratoire aérobie est demeuré constant chez les deux groupes. M_N était plus faible chez les poissons entraînés, ce qui donnait un quotient azote beaucoup plus bas (et stable); le quotient azote augmentait avec la durée de la nage chez les poissons non entraînés. Les lipides constituaient la principale source d'énergie pour l'exercice aérobie chez les deux groupes. Les hydrates de carbone jouaient aussi un rôle important, tandis que les protéines présentaient une moindre importance quantitative. L'entraînement a provoqué une baisse de la dépendance à l'égard des protéines et une hausse de la dépendance à l'égard du métabolisme des lipides. Le rôle de l'oxydation des protéines pour soutenir la nage aérobie chez les poissons, qui est moindre qu'on ne le croyait généralement, diminue encore après entraînement.
[Traduit par la Rédaction]

Introduction

Exercise training has been studied extensively in fish using a variety of durations (from a few days to more than a year) and swimming intensities (less than 0.5 to 3.0 body lengths per second (BL/s); reviewed in Davison 1989). Many of the studies have focused on changes in muscle characteristics such as fiber size (Davison and Goldspink 1977; Greer Walker and Emerson 1978; Johnston and Moon 1980; Davie et al. 1986; Gamperl and Stevens 1991), sequence of fiber recruitment (Johnston and Moon 1980), as well as distribution and supply of capillaries (Davie et al. 1986). Growth rates (Greer Walker and Emerson 1978; Houlihan and Laurent 1987), body condition factor (Davie et al. 1986; Gamperl and Stevens 1991), food conversion efficiency (Davison and Goldspink 1977), and blood variables (Davie et al. 1986) have also been investigated. The fact that there have been no previous studies on how training of fish might alter fuel utilization during swimming led to the present investigation.

We employed the respirometric methods developed by Lauff and Wood (1996*a*) for studying instantaneous fuel usage in fish. This approach is based on simultaneous measurements of O_2 consumption (M_{O_2}), CO_2 production (M_{CO_2}), and nitrogenous waste excretion (M_N, representing ammonia-N plus urea-N), and the application of standard metabolic theory (Kleiber 1987, 1992). This differs from the traditional compositional approach in measuring the fuels actually metabolized rather than the fuels depleted over time. Using this approach with untrained juvenile rainbow trout at several aerobic

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swimming speeds, Lauff and Wood (1996*b*) demonstrated that lipid oxidation predominated throughout the test period, that protein oxidation was kept at minimum levels necessary for maintenance, and that carbohydrate oxidation increased with swimming speed. The results were notable inasmuch as they tended to contradict the well-established view that proteins dominate in the mix of metabolic fuels (Kutty 1972; Van den Thillart 1986; Davison 1989), that lipids are important as well (Atherton and Aitken 1970; Jezierska et al. 1982; Van Waarde 1983), and that carbohydrate is of minor importance (Black et al. 1962; Van den Thillart 1986).

The goal of the present study was to test whether the surprisingly low reliance on protein (20–36%) and high reliance on carbohydrate (up to 38%) would be altered by prior training. The results of Lauff and Wood (1997) demonstrated that relative protein usage did increase with 3 days of low-speed swimming in previously untrained fish, whereas relative carbohydrate usage decreased. Such trends might be the start of a training effect, which should be better developed after 2 weeks of continuous low-speed swimming. A training speed of 1.0 BL/s was chosen because Greer Walker and Emerson (1978) found this speed to be optimal for growth and muscle hypertrophy. In addition, Weihs (1973) predicted a cruising speed of 1.0 BL/s to be the energetically most efficient swimming speed for salmonids whereas Quinn (1987) showed that migrating sockeye salmon (*Oncorhynchus nerka*) do travel at that speed. A testing speed of 2.1 BL/s was selected on the basis of the consensus that exercise at this speed is powered mainly by aerobic red muscle (Webb 1971; Hudson 1973; Wilson and Eggington 1994; Moyes and West 1995).

Materials and methods

Animals and general protocol

The husbandry, test regime (save for the training, detailed below), respirometry (O_2 and CO_2), water nitrogen assays (total ammonia [T_{amm}], urea), proximate body analyses (protein, carbohydrates, lipids, water, and ash), and calculations for fuel use were exactly as described in Lauff and Wood (1996*b*). The untrained fish of the present study are the low-speed swimmers from that study. Fish were held and trained in dechlorinated Hamilton tap water (see Lauff and Wood (1996*a*) for composition) at 15 ± 1°C. Fish were fed 1% of their body mass per day. After training and prior to respirometry, the average starting mass and fork length of the trained fish (16.5 ± 2.2 g, 112 ± 5.8 mm (mean ± SE); $n = 5$) was not different from that of the untrained fish (19.8 ± 0.8 g, 119 ± 1.8 mm; *t* test, $n = 10$; $p > 0.05$ for both mass and length). The final mass of the trained fish had dropped 5.2 ± 0.9% during respirometry (when they were not fed) whereas that for the untrained fish dropped 4.5 ± 1.0%; the difference between groups was not significant.

Training

The trained fish underwent 2 weeks of swimming at 1.0 BL/s in a Beamish-style swim tunnel (Farmer and Beamish 1969). This speed represented about 25% of the maximum sustainable speed (U_{crit}) in untrained trout (Lauff and Wood 1996*b*). The swim tunnel had a total capacity of approximately 200 L; fresh, dechlorinated Hamilton tap water entered, while mixed water left continuously at 4 L/min. The swim tunnel motor was turned off during feeding to ease access of the food to the fish; feeding took no longer than 5 min each day.

Testing

The fish were quickly blotted (no anaesthesia), weighed, measured, and placed in individual 3.2-L Blaka-style respirometers (Blaka et al. 1960, in Beamish 1978) with a water replacement rate of 150 mL/min. The respirometers were served with decarbonated (not softened) water made from dechlorinated Hamilton tap water as described by Lauff and Wood (1996*a*, 1996*b*). These studies have shown that decarbonation does not affect repiratory gas exchange or aerobic swimming performance but does increase analytical precision for CO_2 measurements. Untrained fish were transferred directly from their holding tank (containing dechlorinated tap water) to the respirometers, whereas trained fish were transferred directly from the Beamish-style swim tunnel (also containing dechlorinated tap water). The fish were allowed to adapt to the respirometers for 2 days at a current of less than 1 BL/s, to which they oriented but did not swim. A continuous swimming speed of 2.1 BL/s (55% of U_{crit} in untrained trout; Lauff and Wood 1996*b*) was utilized for the fuel usage tests in the Blaka respirometers. The fish were not fed while in the respirometers.

Three respirometry periods (each 0.5–1.0 h) were run at 5-h intervals on each of 3 consecutive days (total test duration 58 h). During these periods, the respirometers were sealed, and water samples were taken over time for determination of M_{O_2}, M_{CO_2}, and M_N and thereby calculation of respiratory quotient ($RQ = M_{CO_2}/M_{O_2}$) and nitrogen quotient ($NQ = M_N/M_{O_2}$). At the end of the experiment, the fish were rapidly killed by an overdose of MS-222 (1 g/L), freeze clamped in liquid nitrogen, and stored at –70°C for later analysis of proximate body composition. Total body protein, lipid, inorganics (ash), water, glycogen, glucose, and lactate were measured, and the sum of the latter three has been reported as total carbohydrate.

Calculations and statistics

The instantaneous fuel usage calculations, and the rationale behind them, have been detailed by Lauff and Wood (1996*a*). In brief, they used only aerobic RQ measurements (≤1.0), relied on standard RQ_{lipid} (0.71) and $RQ_{carbohydrate}$ (1.0) constants, an empirically determined $RQ_{protein}$ calculated from the relative contributions of ammonia-N and urea-N to M_N as described by Kleiber (1987), and a theoretical maximum NQ of 0.27 (van den Thillart and Kesbeke 1978) when all aerobic metabolism is fueled by protein. These data allow calculation of fuel usage in terms of the relative contribution of lipid, carbohydrate, and protein fuels to the support of M_{O_2}. In turn, the results may be converted to absolute carbon expenditures from the three fuel types using the fuel-specific RQs and the measured values of M_{CO_2} (Fig. 1).

Data are expressed as means ± 1 SE. Regressions were fitted by the method of least squares and tested for significance using the Pearson linear correlation coefficient and the appropriate *t* test (Fig.P graphics package, Ferguson, Mo.). An independent *t* test was used to evaluate possible differences between treatment groups in gas exchange and in body composition measurements at common sampling times. For all tests, $p < 0.05$ was considered significant.

Results

Respirometry and instantaneous fuel use

In both groups, the initial oxygen consumption of fish swimming at 2.1 BL/s was about 10 $\mu mol \cdot g^{-1} \cdot h^{-1}$. M_{O_2} decreased by the second period and stabilized thereafter ($p > 0.05$) in the trained group but continued to decline ($p < 0.0001$) in the untrained group (Fig. 1). M_{CO_2} was more variable. Overall, there was no change in M_{CO_2} over time in trained fish, whereas it decreased significantly in the untrained fish ($p < 0.001$). Averaged over the entire 58-h regime, the gas exchanges were not significantly different in the two groups.

RQ was somewhat variable in both groups, although values tended to remain between 0.8 and 1.0 (Fig. 2). When anaerobic values (RQ > 1.0) were removed, the aerobic RQ values,

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Fig. 1. Oxygen consumption and carbon dioxide excretion of untrained (circles; $n = 10$) and trained (squares; $n = 5$) juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test. Values are mean ± 1 SE.

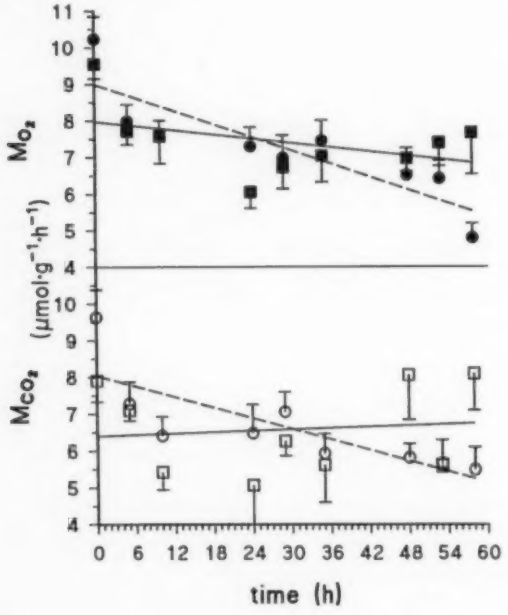
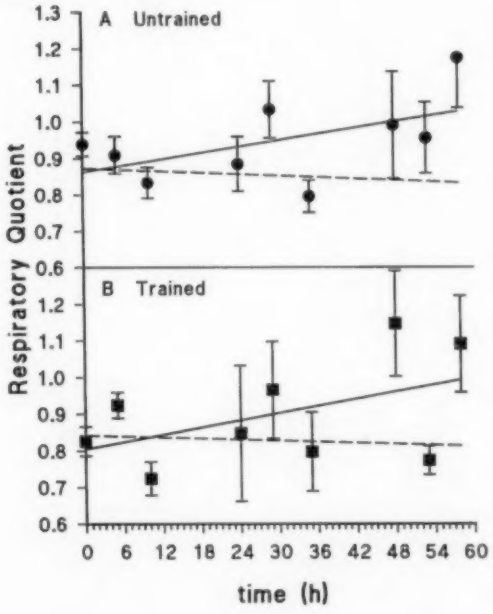


Fig. 2. The complete respiratory quotient (symbols and solid regression line) and the aerobic respiratory quotient (broken regression line) for (A) untrained ($n = 10$) and (B) trained ($n = 5$) juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test. Values are mean ± 1 SE.



replotted as regressions, showed almost identical and stable ($p > 0.05$) lines (Fig. 2).

Nitrogenous waste excretion, except for the final value of the trained group, was stable ($p > 0.05$) over the 3-day test period (Fig. 3). However, the untrained group had an average excretion rate of 0.54 μmol $N \cdot g^{-1} \cdot h^{-1}$ (made up of 18% urea, 82% T_{amm}), while the trained fish showed an excretion rate 39% lower at 0.33 μmol $N \cdot g^{-1} \cdot h^{-1}$ (23% urea, 77% T_{amm}; $p < 0.01$ for the lower rate, $p < 0.0001$ for the difference in composition). On the basis of these urea-N:ammonia-N ratios, we calculated that the $RQ_{protein}$ was 0.95 for the untrained group, while for the trained fish, the value was 0.94. These data permitted calculation of instantaneous fuel usage.

NQ remained effectively stable ($p > 0.05$) at 0.04 in the trained group but increased linearly ($p < 0.001$; $r = 0.418$) from 0.06 to over 0.11 in the untrained group (Fig. 4). Given that aerobic protein catabolism is proportional to nitrogenous waste excretion, the right axis of Fig. 4 allows interpretation of this variable. The relatively stable NQ of the trained group arose from a protein use that represented 17% of the total fuel mixture. The protein use in the untrained group was greater than in the trained fish ($p < 0.02$) and rose steadily ($p < 0.001$) from 22% to greater than 35% at the end of the final day.

The patterns of lipid and carbohydrate usage were similar in the trained and untrained fish, with the contribution of the former increasing and that of the latter decreasing over time (Fig. 5). Lipid was always responsible for greater than half the fuel used in the trained group and showed a final contribution of 60%. The contribution of lipid to the total fuel mixture in the trained fish was consistently 10% higher than in the untrained group. In contrast, carbohydrate initially played a

Fig. 3. Total nitrogenous waste (sum of ammonia-N and urea-N; solid symbols) and ammonia-N (open symbols) excretion for (A) untrained ($n = 10$) and (B) trained ($n = 5$) juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test. Values are mean ± 1 SE.

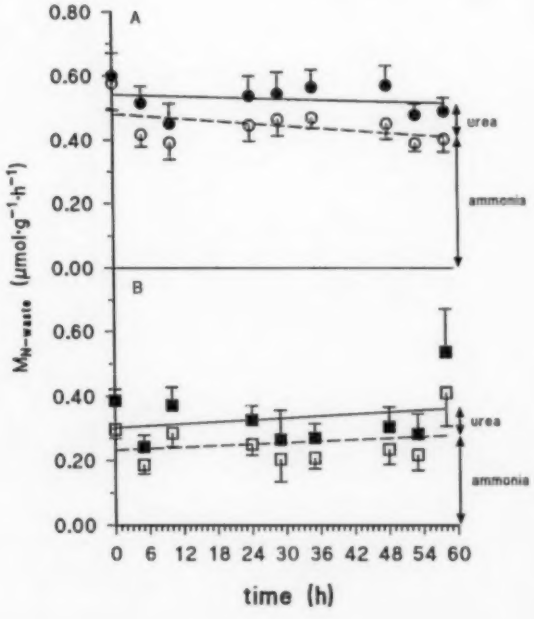


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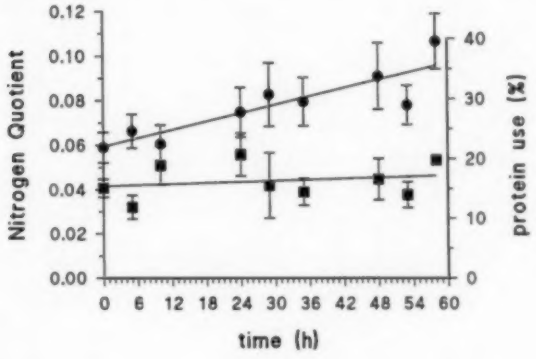
Fig. 4. Nitrogen quotients (left axis) and protein use (right axis) for untrained (circles; $n = 10$) and trained (squares; $n = 5$) juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test. Values are mean ± 1 SE.



stronger role in the untrained fish, representing an average of 37% on the 1st day, but the contribution decreased to 14% on the final day; in the trained group, carbohydrate usage also decreased over time but with a narrower range of 34–21%.

In both groups, the carbon use rate from protein remained relatively constant, although the average use rate in the trained fish was significantly lower at 15 μg $C \cdot g^{-1} \cdot h^{-1}$ ($p < 0.0001$) than the value of 25 μg $C \cdot g^{-1} \cdot h^{-1}$ in the untrained fish. Carbon use rate from carbohydrate in trained trout decreased initially and then levelled off at about 23 μg $C \cdot g^{-1} \cdot h^{-1}$, whereas it declined continuously in the untrained group from 49 to 7 μg $C \cdot g^{-1} \cdot h^{-1}$. Finally, carbon use rate from lipid started at similar values in the two groups (40 μg $C \cdot g^{-1} \cdot h^{-1}$) but thereafter declined and then stabilized at 30 μg $C \cdot g^{-1} \cdot h^{-1}$ for the duration of the experiment in untrained fish. In contrast, the burning of lipid carbon was typically higher in the trained fish, with a slight tendency to increase on the final day.

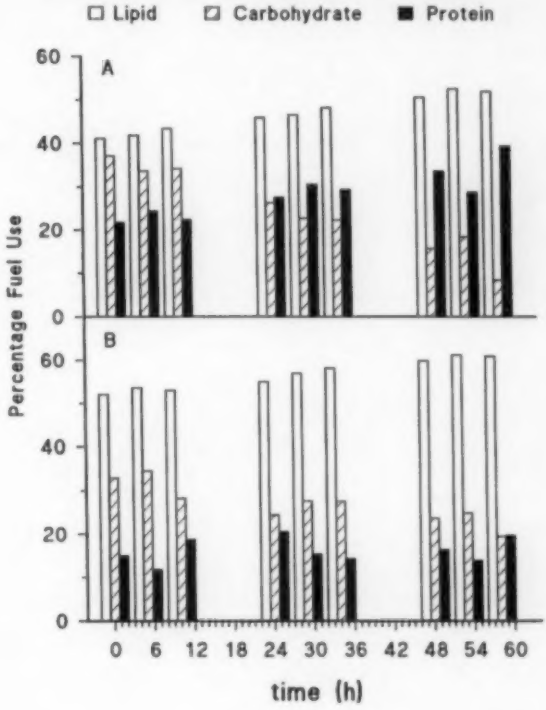
Body composition

Terminal concentrations of lipid, inorganics (ash), and water of trained and untrained fish were similar ($p > 0.05$; Table 1). Both protein and total carbohydrate levels were higher ($p < 0.025$ and 0.05, respectively) in the trained fish than in the controls.

Discussion

In this study, some of the measured variables were strongly affected by training, whereas others were not. There was, in fact, a dichotomy between the traditional respiratory variables (M_{O_2}, M_{CO_2}) and N-waste excretion. For the former, even though the trained fish did not show the decline in respiratory gas exchange that was present in the untrained group, there was no difference in absolute amount of gas exchange between groups (Fig. 1). However, decreased N-waste excretion and resulting fuel use differences were obvious (Figs. 3 and 4). Because the total amount of O_2 consumed and the total amount of carbon used by the trained group were not lower than that of the untrained fish, we suggest that no greater efficiency of the conversion of fuel energy to locomotory work occurred as a result of the training regime imposed. The changes that did

Fig. 5. Percentage use of lipid (open bars), carbohydrate (hatched bars), and protein (solid bars) in (A) untrained and (B) trained juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test.



occur were the result of substantially lower protein catabolism, which was reflected in the lower N excretion (Figs. 3 and 4).

To satisfy the assumptions of the analysis (Lauff and Wood 1996*a*), apparent anaerobic values of RQ (>1.0) were not used in the calculation of fuel use. If some of these bouts were due not to anaerobiosis but rather to lack of steady-state conditions in the fish, omitting these data would increase the apparent contribution of lipid while decreasing that of carbohydrate; the contribution of protein would not be affected. Therefore, no error would result in the principal conclusion of this study, that training results in a decreased reliance on protein in fueling aerobic exercise.

Nitrogenous waste and protein use

This conclusion is opposite to the speculation of the Introduction that the gradual increase in relative protein use by the untrained fish over the 3-day test period (as seen in Fig. 4) might be the start of a training effect. Rather, it is clear that the trained fish relied to a lesser extent on protein as a fuel. In hindsight, this strategy appears entirely reasonable. Unlike lipid or carbohydrate, which can be stored in readily mobilizable deposits, protein is largely stored as the contractile material of muscle. For continuously exercising fish, it makes little sense to burn the very machinery that is powering the swimming. Instead, training reorganizes metabolism so as to spare the muscle.

In accord with this argument, terminal body protein content was significantly higher in the trained fish (Table 1), which

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Table 1. Body compositions (mg/100 mg, wet mass; mean ± SE) of untrained and trained juvenile rainbow trout after exercise.

	Lipid	Total carbohydrate	Protein	Inorganic	Water
Untrained fish	5.1±0.7	0.20±0.02	13.2±0.7	2.68±0.17	77.8±1.2
Trained fish	5.0±0.1	0.25±0.02*	15.3±0.4**	2.42±0.11	77.5±0.5

Note: Total carbohydrate includes glucose, glycogen, and lactate. *, $p < 0.05$; **, $p < 0.025$ versus untrained fish.

reflected the well-documented hypertrophy of one or both muscle types during training (Greer Walker and Emerson 1978; Johnston and Moon 1980; Davie et al. 1986; Houlihan et al. 1986; Houlihan and Laurent 1987; Gamperl and Stevens 1991). Interestingly, Lauff and Wood (1996*b*) found that high-speed swimmers (3.1 BL/s), the most exercised group in that study, used the lowest percentage of protein as a fuel. The trained group in the present study had gone directly from a 2-week training regime to the swimming test. Thus, in both cases, the fish subjected to the greatest amount of exercise, either in terms of duration or intensity, relied less on protein as a fuel than their less-exercised counterparts.

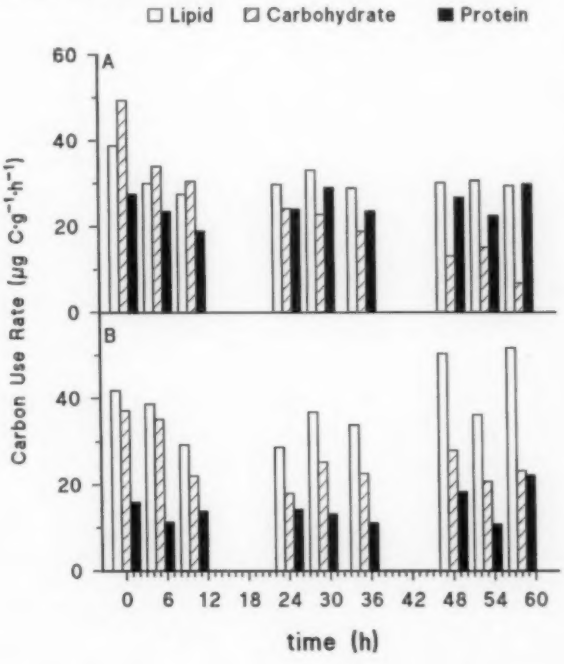
Lipid use

Because the total amount of carbon burned was similar in the two groups (Fig. 1, CO_2 excretion), and the total carbon originating from protein was lower in the trained fish (Fig. 6), then the prediction would be a simple increase in the contribution of either or both of the other two fuels to make up the balance. In fact, the contribution of lipid, the most efficient fuel on a per mass basis (Kleiber 1992) was consistently higher in the trained fish. This did agree with the increasing reliance on lipid exhibited by the untrained fish during their first 3 days of low-speed swimming (Fig. 5). The conclusion also agrees with the generally accepted view that lipids, specifically free fatty acids, are important fuels for long-term aerobic swimming (Driedzic and Hochachka 1978*a*; Mommsen et al. 1980; Walton and Cowey 1982; Henderson and Tocher 1987; Greene and Selivonchick 1987). However, the mechanism(s) by which lipids are mobilized from muscle and other depots in fish remain(s) ill defined (Henderson and Tocher 1987; Greene and Selivonchick 1987). Even though lipid use was higher in the trained fish (Figs. 5 and 6), there was no difference between the two groups with respect to the final body content of lipid (Table 1). Either the 3-day test period was too short to detect differences in depletion, or there was more lipogenesis (or higher lipid stores at the end of the training period and prior to the test) in the trained fish. Certainly, the control of lipid metabolism during aerobic exercise is a rich field for further investigation, as are mechanisms involved in the transition of fuel use caused by training.

Carbohydrate use

The contribution of carbohydrate in the trained fish was at or below the contribution in the untrained group seen over the 1st day of the test period. By the 3rd day, the untrained fish continued to decrease their reliance on carbohydrates such that the contribution fell below that of the trained fish. Nevertheless, the trained fish had larger carbohydrate stores than the

Fig. 6. The contribution of carbon from lipid (open bars), carbohydrate (hatched bars), and protein (solid bars) in (A) untrained and (B) trained juvenile rainbow trout swimming at 2.1 BL/s over the 3-day test.



untrained fish at the end of the 58-h swim test (Table 1). Earlier, Scarabello et al. (1991) showed that carbohydrate stores are very labile in untrained trout of this size, especially in the face of prolonged exercise and starvation. Furthermore, Johnston and Moon (1980) reported a twofold increase in white muscle and a fivefold increase in red muscle glycogen levels after 3 weeks of training at 2.1 BL/s in pollack (*Pollachius virens*).

The reasons for these carbohydrate patterns are unclear. Carbohydrate is typically thought of as the fuel of the white muscle (Johnston and Goldspink 1973; Driedzic and Hochachka 1978*a*, 1978*b*; Mommsen et al. 1980). The white muscle is considered by many to function primarily in burst swimming (Weihs 1973), and it is reasonable to assume that red muscle activity predominated in the present tests at 55% U_{crit} (see Introduction). Nevertheless, it has been shown electromyographically that the mosaic fibers may become active at speeds as low as 35% U_{crit} in rainbow trout (Hudson 1973). It therefore seems probable that the fish in the current study were utilizing white muscle to some degree for their swimming at 55% U_{crit}. Unfortunately, no study has investigated differential recruitment of muscle fiber types as a result of training.

Conclusions

The main effect of training we observed was a decrease in protein catabolism during aerobic swimming (reflected in reduced N excretion), and an associated increase in lipid use, though the total amount of fuels used was unchanged. Carbohydrate usage remained appreciable. If trained trout are

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representative of wild fish, then salmonids in nature may burn very little protein indeed.

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Eutrophication, pollution, and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland

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Abstract: Parasite communities in four study lakes in 1986 reflected the influences of eutrophication, pollution, and habitat fragmentation. Discriminant analyses of communities at the individual host level revealed two major axes. One contrasted communities in a lake affected by chemical pollution from a pulp mill with those from two eutrophic, less polluted lakes. Changes in the density of intermediate hosts, direct effects on ectoparasites, and impaired immune systems were regarded as important mechanisms. The second contrasted communities in an oligotrophic, unpolluted lake with those from the two eutrophic lakes and was more complex, reflecting habitat fragmentation (the absence of glochidia and some digeneans) and pollution or eutrophication, probably mediated by the same mechanisms as above. Changes in some index parasites in Lake Vatia monitored in 1994, following 8 years of reduced pollutant loading, supported our conclusion that parasite faunas in Lake Vatia in 1986 involved the effects of pollution.

Résumé : Les communautés de parasites examinées dans quatre lacs en 1986 révélaient les influences de l'eutrophisation, de la pollution et de la fragmentation de l'habitat. Des analyses discriminantes des communautés au niveau individuel des hôtes ont fait ressortir deux grands axes. Le premier comparait les communautés d'un lac affecté par la pollution chimique due à une usine de pâte à papier à celles de deux lacs eutrophes moins pollués. Les changements dans la densité des hôtes intermédiaires, les effets directs sur les ectoparasites et l'affaiblissement des systèmes immunitaires ont été considérés comme des mécanismes importants. Le second axe comparait les communautés d'un lac oligotrophe non pollué à celles des deux lacs eutrophes, et il présentait une plus grande complexité, qui traduisait la fragmentation de l'habitat (absence de glochidiums et de certains digéniens), ainsi que la pollution ou l'eutrophisation, qui se manifestent probablement par les mêmes mécanismes que ci-dessus. Des changements observés chez certains parasites indicateurs du lac Vatia surveillé en 1994, après 8 ans de réduction de la charge en polluants, nous permet de conclure que la faune des parasites du lac Vatia en 1986 reflétait les effets de la pollution.
[Traduit par la Rédaction]

Introduction

Eutrophication and chemical pollution are problems facing many freshwater ecosystems throughout the world. In addition, some systems are also affected by new barriers to fish movement, hence increased fragmentation of the habitat. Although the consequences of these three processes for fish and to a lesser extent for free-living invertebrates are reasonably well understood, their effects on parasite faunas are not.

Most of the literature on the effects of eutrophication on parasites has in fact examined the parasite communities in lakes of different trophic status. Many such studies concluded that lakes of higher trophic status have more individual parasites and more and different species of parasites (see references in Moser and Cowen 1991). Wisniewski (1958) and Esch (1971) also concluded that the parasite communities in fish in eutrophic lakes are dominated by allogenic species (larval stages of species maturing in birds or mammals), rather than the autogenic species (adult parasites or those maturing in fish) that characterize oligotrophic lakes. Others, however (e.g., Halvorsen 1971; Wootten 1973; Kennedy 1978), found no relationship between fish parasite communities and lake trophic status and emphasized the importance of the history of, and conditions in, individual lakes.

However, such studies may have little importance in understanding the influence of anthropogenic eutrophication, where the magnitude and speed of change often surpasses the ability of the system to adapt (Rapport et al. 1985). Longitudinal studies on systems during a period of eutrophication have shown that minor increases in nutrients, and hence productivity, may increase the numbers and kinds of parasites in the system (Hanek and Fernando 1978; Kennedy 1985; Moser and Cowen 1991). Such changes can have marked effects on fish populations. Hartmann and Nümann (1977) reported that eutrophication in Lake Constance led to increases in diplostomids, which caused periodic early summer kills of adult perch (*Perca fluviatilis*). More extensive nutrient loading may produce the

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same results as pollution stress (Regier and Cowell 1972), i.e., either decreases (Kennedy 1993) or increases (Marcogliese et al. 1990) in parasite numbers; the latter may even initiate extensive changes in the fish community (Kennedy et al. 1994).

The influences of chemical pollution on parasites can be complex, because of the multiple ways that such pollution may affect parasites (see review by Khan and Thulin 1991). Chemicals may have direct effects on the free-living or ectoparasitic stages of the parasite or may act through changes in the density of one or more required hosts (Hirschfield et al. 1983); changes in the extent to which those hosts overlap in distribution (Marcogliese et al. 1990); or changes in the physiology, particularly the immune response, of the fish (Jokinen et al. 1995). In addition, conditions favoring parasites but impairing fish condition may induce disease, particularly in the more heavily infected fish (see reviews by Snieszko 1974; Khan and Thulin 1991). Theoretically, at least, such situations may control parasite populations (Scott and Dobson 1989), obscuring the direct effects.

Sprent (1992) suggested that human activities, including those leading to habitat fragmentation, pose a serious threat of extinction to ascaridoids (and, by extension, to other parasites). Although Sprent was particularly concerned with the threatened extinction of the hosts, the reduced local host population sizes stemming from increased habitat fragmentation may pose an independent threat of at least local parasite extinction (see the theoretical model of parasite richness as a function of host population size in Dobson and Pacala 1992). Reviewing this topic, Bush and Kennedy (1995) acknowledged that extinction of local metapopulations may occur but consider that parasites are well adapted for transmission, that many of those adaptations function as "hedges against extinction," and that reinvasion from other metapopulations (a "rescue effect") is likely to prevent global extinction, so long as none of the required hosts becomes extinct. Freshwater systems are already extensively fragmented. At a local level, this fragmentation, plus the limited dispersal capacity of freshwater fishes and their autogenic parasites, has been considered one of the major features leading to the low numbers of parasite species characteristic of freshwater systems (Kennedy et al. 1986; Esch et al. 1988; Kennedy 1990). This may be evidence that local rescues are rather ineffective, at least over several decades. Additional fragmentation, through erection of barriers to fish movement between connected lakes, may therefore contribute to further reductions in species richness.

Kennedy (1978) and Hartvigsen and Kennedy (1993) stressed that the individual history of each lake and the individual characteristics of that lake are the most important factors determining the parasite communities in brown trout (*Salmo trutta*) in lakes in Great Britain, and in reservoirs in southwestern England, respectively. However, R. Hartvigsen and O. Halvorsen (unpublished data) have shown that natural lakes in Norway connected by the same tributary stream have parasite assemblages that are almost as similar as samples from different parts of the same lake.

Most of the studies on eutrophication, pollution, and fragmentation have compared lakes with different histories and different conditions. Central Finland is characterized by numerous large lakes, organized into extensive southward-draining chains, connected by broad, short, low-relief rapids. Tonn et al. (1990, and references therein) concluded that there were two major historical reasons why even small Finnish lakes have a much higher proportion of the available fish fauna than comparable lakes in Wisconsin. First, because of a lack of nearby glacial refuges, conditions in northern Europe selected for larger, longer lived, and migratory species of fishes, with broader habitat-usage patterns. Second, all lake basins are remnants of the postglacial Lake Ancylus, ca. 6500–6000 BC, in which most species of the modern regional fauna already lived; most species remained where they had been when isostatic rebounding lifted the land and produced the chains of lakes.

In Central Finland, therefore, we had the opportunity to investigate a series of interconnected lakes, with as nearly similar histories and conditions as can be found, which have been subject to the action of pollution, eutrophication, and increasing fragmentation over the past 50 years. Since the 1950s, the lakes have been separated by dams and other obstacles, and three have been exposed to various combinations of eutrophication and pollution.

The parasites of the perch and roach (*Rutilus rutilus*) in these lakes have been the subject of an extensive research program, much of it published previously (Brummer-Korvenkontio et al. 1991; Koskivaara et al. 1991*a*, 1991*b*, 1992; Koskivaara and Valtonen 1991, 1992; Halmetoja et al. 1992; Tuuha et al. 1992; Valtonen et al. 1993, 1994; Haaparanta et al. 1994; Valtonen and Koskivaara 1994). Our goal here is to examine overall patterns in the parasite communities in these two fishes in the four lakes during 1986, focussing particularly on patterns at the individual fish (infracommunity) and fish population (component community) levels. In addition, we were able to check some of our conclusions on the effects of chemical pollution, using data from a monitoring study conducted on three of the lakes by M. Koskivaara in 1994, following a period of recovery.

Study area

The study area consists of four lakes in the Viitasaari chain of lakes, draining via Lake Päijänne to the Gulf of Finland (Fig. 1). Three of these lakes are part of the main drainage system; the fourth is an isolated tributary. The lakes are ice covered from late November through mid-May, are stratified during the summer, and reach their highest temperatures, 18–20°C, in late July or August. Lakes Vatia and Saravesi are semilotic, with significant currents and short water retention times (Table 1). Some other basic characteristics of the lakes are also given in Table 1.

Prior to the 1920s, fish migration among the lakes was impeded only by rapids between lakes. Electricity plants built in the 1920s between lakes Saravesi and Leppävesi and between lakes Leppävesi and Päijänne almost stopped upstream migrations of fish (catch statistics of the fishway: V. Honkanen, Fisheries Section, Rural Business District of Central Finland, personal communication). In addition, construction of road and railroad embankments, with limited viaducts, in the late 1940s provided an obstacle to fish movement between southern and northern Lake Leppävesi. A dam was built between lakes Peurunka and Vatia in the late 1960s specifically to prevent upstream movement of fish. These barriers largely fragmented this chain of lakes, isolating Lake Peurunka and to a

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large extent the other lakes. Lake Saravesi remained connected to the oligotrophic Lake Kuusvesi.

Three of the lakes (Vatia, Saravesi, Leppävesi) have been influenced since the 1940s by nutrient loading from pulp mills in the city of Äänekoski, 15 km upstream from Lake Vatia (Fig. 1). Chlorine bleaching of pulp started in 1956, and the quantity of organochlorine compounds in the waters of the recipient lakes soon increased over the background level (Paasivirta et al. 1976). During the following 30+ years the downstream lakes Kuhnamo, Vatia, Saravesi, and Leppävesi and the northern parts of Lake Päijänne were heavily influenced by the effluent from these factories. The loading of Äänekoski pulp mills as measured by biological oxygen demand (BOD_7) was between 55 and 70 t·day^{-1} at the beginning of the 1970s. During this period, a summertime lack of oxygen was a yearly occurrence in Lake Vatia (Granberg 1992).

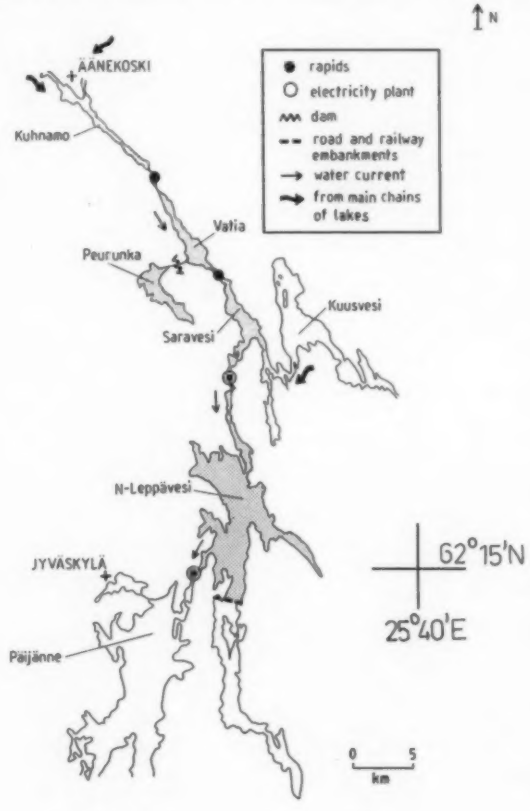
The quality of benthic communities differed greatly between lakes Vatia and Leppävesi, 69 and 96 benthic taxa being found in the lakes, respectively. Pollution-tolerant chironomids and oligochaetes were present in exceptionally high abundances in Lake Vatia, but among molluscs, only three Sphaeridae species (*Sphaerium corneum* and two *Pisidium* species) were found in Lake Vatia in 1986, while in Lake Leppävesi, seven small bivalve and four snail species were encountered (Hynynen 1987; Hynynen and Meriläinen 1987). The ratio of zooplankton species describing eutrophy and oligotrophy was highest in Lake Vatia (2.52), while the same ratio in Lake Leppävesi was 1.13 and in the unpolluted reference Lake Kuusvesi was 0.59 (Palomäki and Bibiceanu 1987).

Catch statistics showed that all four lakes had the same species of fish, with good populations of roach, perch, pike (*Esox lucius*), and burbot (*Lota lota*). The main differences among lakes in 1986 were that whitefish (*Coregonus lavaretus* sensu lato) and vendace (*Coregonus albula*) were common in lakes Peurunka and Leppävesi but scarce in lakes Saravesi and Vatia; bream (*Abramis brama*) were common in all except Lake Peurunka; and zander (*Stizostedion lucioperca*) were common only in lakes Saravesi and Leppävesi.

Water quality started to improve in 1985, as a result of a change in technology. In 1986, when the present study was done, the pulp mill effluent loading was 4.3 t·day^{-1} (BOD_7) and 2000 kg·day^{-1} AOX (adsorbable organic halogens); as a result, in 1986 no oxygen deficiency was found in the epilimnion of Vatia at any time of the year (Granberg 1987). The use of gaseous chlorine in bleaching was stopped in 1991; by 1994, BOD_7 loading was down to 2.7 t·day^{-1} and AOX down to 500 kg·day^{-1} (K. Granberg, University of Jyväskylä, Jyväskylä, Finland, personal communication). As a result, lakes Saravesi and Leppävesi became mesotrophic, using the criteria of Forsberg and Ryding (1980), and pollution in these lakes was ameliorated, as indicated by AOX, profundal biomass, and benthic quality index (Table 1). In addition, by 1994, zander and the coregonids were increasing in both lakes Saravesi and Vatia.

In summary, during our study period, Lake Peurunka was isolated, oligotrophic, and unpolluted; there was no significant change in conditions by 1994. Lake Vatia was isolated, significantly polluted, and eutrophic; by 1994, the degree of pollution was decreasing, but it was still eutrophic. Lake Saravesi was not isolated, was somewhat polluted, and was eutrophic; by 1994, it was recovering from pollution and mesotrophic. Lake Leppävesi was isolated, slightly polluted, and eutrophic; by 1994, it was recovering from pollution and mesotrophic. Lakes Vatia, Saravesi, and Leppävesi had been considerably more polluted in previous years.

Fig. 1. The study area in central Finland. The four study lakes are shaded; connections to other parts of the chains of lakes and obstacles to fish migration are indicated. Lake Peurunka is oligotrophic and unpolluted, Lake Vatia is eutrophic and significantly polluted, and lakes Saravesi and Leppavesi are eutrophic or mesotrophic and have decreasing levels of pollution.



Materials and methods

Fifteen perch and 15 roach were sampled from each lake, by angling, monthly (bimonthly during the period of ice cover) throughout 1986. Fish were taken to the laboratory alive in lake water immediately after being caught and were studied within a few hours. A total of 459 roach and 409 perch were examined for macroparasites, using the methods developed by V.A. Dogiel (Bykhovskaya-Pavlovskaya 1985). Sample sizes for some organs or tissues were slightly lower, but 394 roach and 341 perch were examined for all parasites. All fish were adults; size ranges were similar, except that roach from Lake Peurunka were somewhat larger, and perch from Lake Leppävesi somewhat smaller, than those from the other lakes.

Each fish was killed by severing the spinal cord. Slime samples, taken from the first gill arch and from approximately 2 cm^2 under the pectoral fin, were examined at 100× magnification for protozoans. All fins were excised, the rays were split, and they were examined with transmitted light at 25–40×. The gills from one side were excised and examined for large parasites, then the soft tissue was scraped off and

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Table 1. Characteristics of the four study lakes in Central Finland.

	Year	Lake Peurunka	Lake Vatia	Northern Lake Saravesi	Northern Lake Leppävesi	Reference
Elevation (m)		105.9	89.0	84.7	80.8	Topographical maps
Area (km^2)		6.5	5.5	4.7	36.0	Granberg 1987
Mean depth (m)		8.6	4.0	3.0	11.0	Granberg 1987
Volume ($m^3, \times 10^6$)		61.5	22.6	12.2	386.0	Granberg 1987
Water retention time (days)		3.4 years	3	2	32	Granberg 1987
Total P ($\mu g \cdot L^{-1}$)	1986	na	51.5	35	20	Granberg 1987
	1994	11	36	35	21	Hökka and Rantala 1995; CFPEC[a]
Total N ($\mu g \cdot L^{-1}$)	1986	na	715	610	522	Granberg 1987
	1994	370	613	506	423	Hökka and Rantala 1995; CFPEC[a]
pH	1986	na	6.4	6.6	6.7	Granberg 1987
	1994	7.0	6.9	7.0	6.6	Hökka and Rantala 1995; CFPEC[a]
Colour (mg $Pt \cdot L^{-1}$)	1986	na	70.5	59.0	45.8	Granberg 1987
	1994	15–34	86.0	81.0	40.0	Hökka and Rantala 1995; CFPEC[a]
AOX[b] ($\mu g \cdot L^{-1}$)	1986	na	173	na	115.0	Granberg 1992
	1994	na	107.4	na	48.2	Hökka and Rantala 1995
Chlorophyll *a* ($ng \cdot m^{-3}$)	1986	na	12.5	na	9.99	Granberg 1987
	1994	3.0	8.4	na	5.8	Hökka and Rantala 1995; CFPEC[a]
Profundal biomass >10 m ($g \cdot m^{-2}$)	1986	na	3.5	na	1.5	Hynynen and Meriläinen 1987
	1994	na	1.4	na	1.2	Hökka and Rantala 1995
BQI[c] >10 m	1986	na	0.75	na	2.8	Hynynen and Meriläinen 1987
	1993	na	1.5	na	2.95	Meriläinen and Hynynen 1994

Note: na, not available. Values of N, P, pH, colour, and chlorophyll *a* are averages from May to September for the year indicated.

[a]Water quality statistics, Central Finland Provincal Environmental Centre (unpublished data).

[b]Adsorbable organic halogens.

[c]Benthic quality index (Wiederholm 1980) using a scale from 0 (anoxic) to 5 (oligotrophic).

examined at 25–100×. A sample of muscle tissue (approximately 1 cm^2), taken from behind the pectoral fin, was pressed between glass plates and examined for protozoan cysts. Internal organs were separated to individual Petri dishes. Solid organs were cut into pieces (maximum size $1 \times 0.5 \times 0.5$ cm), pressed between plates, and examined. The gastrointestinal tract was separated into stomach, intestine (cut into pieces), and rectum; each was examined for large parasites, the mucosa was scraped, and both the scrapings and the gut wall were pressed between plates and examined.

All parasites were identified and counted (including *Ichthyophthirius multifiliis* and *Dermocystidium percae* cysts; other protozoans were evaluated semiquantitatively on a 0–3 scale, where 1 is 1–9, 2 is 10–30, and 3 is more than 30 individuals per sample). Some parasites (dactylogyrids, gyrodactylids, trichodinids) were determined to the species level only in the first five fish from each sample (see Koskivaara et al. 1991*a*, 1991*b*; Halmetoja et al. 1992); here we use only total numbers, available for all fish and shown by those authors to be adequate to show overall patterns. Representative samples and any specimens whose identity was not clear were collected and prepared by standard procedures (see references in the primary papers from this study).

Component communities (all those parasites in one species of fish in one lake) were characterized by species richness (total number of taxa recovered), by number of common taxa (>50% prevalence), and by the prevalences and abundances of taxa present. Parasite taxa were classified as common, intermediate (10–50%), or rare (<10% prevalence). Qualitative similarities between component communities were measured by the Jaccard index and quantitative similarities by percent similarity (Krebs 1989), calculated from prevalence data (Holmes and Podesta 1968). Component community diversity was described by the reciprocal of Simpson's index (1/SI), with p_i values calculated from prevalence data. This index emphasizes the common species (Krebs 1989). The use of prevalences (and grouped taxa) gives higher levels of diversity and similarity than when using numbers of parasites; our values are comparable within our study, but not necessarily with those of other studies. Differences in prevalence were tested for significance using the maximum likelihood G test; differences in abundances were tested by the Kruskal–Wallis nonparametric analysis of variance, using programs in SYSTAT (Wilkinson 1990). Unless otherwise stated, significance is at the 0.05% level, using Bonferroni corrections for multiple testing.

Infracommunities (all parasites in an individual fish) were described by number of taxa present, number of parasites, and diversity (1/SI, calculated from intensity data; data on gill parasites were those from one side only, data on protozoans (except *Ichthyophthirius* and *Dermocystidium*) were index values). Differences were tested for significance ($p < 0.05$) with the Kruskal–Wallis test, followed by nonparametric multiple comparison tests (Zar 1984). Patterns of similarity were investigated with a discriminant function analysis, calculated from log-transformed intensity data, using the program in BMDP (Dixon 1990).

Our primary data come from 1986. However, the use of parasites in a government-mandated program monitoring pollution in this group of lakes in 1994 gave us an opportunity to check our conclusions on the importance of the effects of pollution in the 1986 data. In the 1994 monitoring study, the gastrointestinal tract was not examined, and other organs were examined only for the more abundant parasites. Approximately 15 roach and 15 perch were collected per sample period in 1994, from each of lakes Peurunka, Vatia, and Saravesi, using the same sample periods (and basic procedures) as in 1986, providing a total of 326 roach and 345 perch. Here, we use prevalence data on 10 species identified as important in our analysis of the 1986 data (see Results) to test our conclusions on the effects of pollution on the parasites in Lake Vatia. A more complete comparative study is currently in process and will be published separately.

We use ecological terms as defined by Margolis et al. (1982).

Results

A total of 56 taxa (species or species groups) were found in the

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Table 2. Prevalence of parasites in roach from four lakes in central Finland, 1986.

Species[a]	Organ[b]	Prevalence (%) Lake Peurunka (n = 123)	Lake Vatia (n = 93)	Lake Saravesi (n = 119)	Lake Leppävesi (n = 120)	Significance[c]
Protozoa						
Capriniana piscium	G	—	1.1	—	0.8	
Zschokkella nova	GB	19.4	3.9	5.3	16.5	<0.0025
Chloromyxum sp.	GB	0.9	2.6	7.5	3.9	
Trichodinids	G,F,S	2.4	10.8	11.8	6.7	
Apiosoma sp.	G	0.8	7.5	3.4	5.8	
Epistylis sp.	F,S	—	—	0.8	—	
Ichthyophthirius multifiliis	G,S,F	11.4	27.2	37.8	21.7	<0.0025
Myxobolus pseudodispar	M	55.8	42.9	58.0	55.8	
Myxobolus muelleri	K	17.1	34.4	22.7	24.2	
Myxidium rhodei	K	62.6	77.4	79.8	72.5	
Myxobolus spp.	G	7.3	8.7	9.2	18.3	
Rotifera						
Encentrum kozminskii	F	1.6	—	0.8	11.7	
Monogenea						
Dactylogyrus spp.	G	93.5	100.0	88.1	91.7	<0.0025
Gyrodactylus spp.	F,S,G	11.4	13.0	14.4	25.8	
Paradiplozoon homoion	G	8.1	3.2	9.2	9.2	
Trematoda						
Rhipidocotyle fennica l	F	—	20.4	92.4	95.0	<0.0025
Rhipidocotyle campanula l	G	—	15.1	52.9	70.0	<0.0025
Tylodelphys clavata l*	VB	88.2	12.9	62.2	71.4	<0.0025
Tylodelphys podicipina l*	VB	2.4	—	0.8	0.8	
Diplostomum gasterostei l*	VB	70.6	8.6	18.5	51.3	<0.0025
Diplostomum spathaceum l*	E	94.9	58.1	85.6	91.7	<0.0025
Allocreadium isosporum	I	25.9	—	18.3	2.6	<0.0025
Sphaerostoma globiporum	I	—	—	10.6	31.3	<0.0025
Phyllodistomum folium	U	15.5	10.7	4.2	7.6	
Cestoda						
Ligula intestinalis l*	C	—	—	1.7	0.8	
Proteocephalus torulosus	I	1.6	2.2	0.8	1.7	
Caryophyllaeus laticeps	I	9.3	5.1	4.8	7.8	
Nematoda						
Raphidascaris acus l	C,I	38.9	71.8	63.5	63.8	<0.0025
Pseudocapillaria sp.	I	4.9	—	—	0.8	
Thwaitia sp.	G,F	3.3	11.8	2.5	6.7	
Acanthocephala						
Neoechinorhynchus rutili	I	15.7	19.2	5.8	11.3	
Acanthocephalus lucii	I	2.8	1.3	1.0	3.5	
Acanthocephalus anguillae	I	—	12.8	5.8	8.7	<0.0025
Arthropoda						
Ergasilus briani	G	13.8	16.3	14.3	10.0	
Neoergasilus japonicus	F	5.7	21.7	15.1	20.0	<0.0025
Argulus foliaceus	S,F,G	4.1	2.2	7.6	5.0	
Mollusca						
Glochidia l	G,F	—	3.3	11.8	9.2	<0.0025
Hirudinea						
Piscicola geometra	S,F	—	—	2.5	—	

[a]l, larval stage, *, allogenic parasite (see text).
[b]G, gills; F, fins; S, skin; M, muscles; C, coelome; I, intestine; L, liver; K, kidney; VB, vitreal body; GB, gall bladder; U, ureter; E, eye lens.
[c]Bonferroni-corrected.

two species of fish, 38 in roach (Table 2), and 42 in perch (Table 3); abundances of parasites reaching a level of 1.0 in at least one lake are shown in Table 4. Only 24 taxa (17 species and 7 species groups) were found in both species of fish, and only two of those, both digenean metacercaria, were common in both fish. Although parasite communities in the two fish were different and were analyzed separately, the basic patterns were similar, as indicated by the analyses below.

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Table 3. Prevalences of parasites in perch from four lakes in central Finland, 1986.

Species	Site	Prevalence (%) Lake Peurunka ($n = 118$)	Lake Vatia ($n = 61$)	Lake Saravesi ($n = 120$)	Lake Leppävesi ($n = 110$)	Significance[a]
Protozoa						
Ichthyobodo necator	S	0.9	—	—	—	
Capriniana piscium	G	8.6	14.9	7.6	10.2	
Apiosoma companulatum	G,F	39.3	66.0	49.2	59.3	
Trichodinids	G,S,F	38.5	25.5	30.5	30.6	
Trichodina urinaria	U	11.7	2.0	1.8	21.1	<0.0025
Ichthyophthirius multifiliis	G,F	—	3.3	2.5	0.9	
Epistylis sp.	F,G	0.9	10.6	13.6	6.5	<0.0025
Henneguya spp.	G,M,GB	36.9	23.5	26.0	46.3	
Myxobolus guyenoty	G	2.5	—	0.8	2.7	
Myxobolus sp.	S	2.9	—	3.4	7.4	
Zchokkella sp.	GB	2.5	—	5.8	0.9	
Myxidium rhodei	L	—	—	3.3	—	
Dermocystidium percae	F	13.6	41.0	19.3	12.2	<0.0025
Microsporidea	VB	3.4	1.6	—	7.3	
Monogenea						
Gyrodactylus gasterostei	F	—	1.6	6.7	1.8	
Ancyrocephalus percae	G	4.2	3.3	6.7	6.4	
Rotifera						
Encentrum kozminskii	G,F	18.8	17.0	15.3	25.0	
Trematoda						
Tylodelphys clavata l*	VB	98.3	8.2	85.8	79.6	<0.0025
Tylodelphys podicipina l*	VB	36.4	3.3	10.0	16.4	<0.0025
Diplostomum gasterostei l*	VB	97.5	34.4	80.0	86.1	<0.0025
Diplostomum spathaceum l*	E	47.5	4.9	28.6	17.3	<0.0025
Ichthyocotylurus variegatus l*	C	55.3	19.7	79.1	55.8	<0.0025
Bunodera luciopercae	I	85.4	70.7	75.2	56.8	<0.0025
Rhipidocotyle campanula	I	—	—	10.5	2.1	<0.0025
Rhipidocotyle fennica l	F	—	—	—	4.6	
Allocreadium isosporum	I	4.2	—	3.3	—	
Cestoda						
Diphyllobothrium latum l*	M	0.9	—	0.9	—	
Triaenophorus nodulosus l	C	32.0	16.4	28.6	52.6	<0.0025
Ligula intestinalis l*	C	—	—	—	0.9	
Eubothrium sp.	I	—	—	2.5	—	
Proteocephalus percae	I	36.9	8.6	17.1	15.8	<0.0025
Nematoda						
Camallanus lacustris	I	1.9	37.9	63.8	31.6	<0.0025
Desmidocercella sp. l	VB	—	4.9	12.5	2.8	<0.0025
Raphidascaris acus l	C	—	6.6	8.3	2.7	
Acanthocephala						
Neoechinorhynchus rutili	I	3.9	3.5	—	—	
Acanthocephalus lucii	I	46.6	56.9	26.7	32.6	<0.0025
Echinorhynchus borealis	I	0.9	—	—	—	
Arthropoda						
Ergasilus sieboldi	G	13.6	9.8	1.7	4.6	<0.0025
Neoergasilus japonicus	F	—	3.3	0.8	—	
Achtheres percarum	G	27.1	—	10.9	6.4	<0.0025
Argulus foliaceus	S,F,G	11.9	21.6	26.9	18.2	
Mollusca						
Glochidia l	G,F	—	31.2	52.9	63.6	<0.0025

Note: Abbreviations are as in Table 2.

[a]Bonferroni-corrected.

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Table 4. Abundances of parasites in roach and perch from four lakes in central Finland, 1986.

Species	Organ	Lake Peurunka	Lake Vatia	Lake Saravesi	Lake Leppävesi	Significance
Roach						
Ichthyophthirius multifiliis	G,S,F	0.1±0.4	1.2±5.3	4.6±13.4	0.5±1.3	<0.001
Myxidium rhodei[b]	K	1.1±1.2	1.8±1.3	1.7±1.3	1.4±1.2	<0.001
Dactylogyrus spp.[c]	G	19.2±18.7	25.5±25.4	17.2±23.9	24.5±35.8	
Gyrodactylus spp.[c]	F,S	0.2±0.6	0.2±0.6	0.3±1.2	1.3±4.3	<0.001
Rhipidocotyle fennica l	F	—	2.3±11.5	51.6±69.2	67.2±102.7	<0.001
Rhipidocotyle campanula l[c]	G	—	0.9±3.1	5.3±9.5	12.6±29.1	<0.001
Tylodelphys clavata l	VB	40.4±45.2	0.3±1.0	10.3±16.9	14.3±23.3	<0.001
Diplostomum gasterostei l	VB	5.4±9.6	0.1±0.4	0.5±1.5	2.1±4.4	<0.001
Diplostomum spathaceum l	E	30.6±44.2	1.7±2.5	8.4±21.8	8.7±12.0	<0.001
Raphidascaris acus l	C,I	1.1±2.5	4.0±6.5	2.1±2.8	5.7±14.6	<0.001
Perch						
Apiosoma companulatum[b]	G,F	0.6±0.8	1.4±1.5	1.0±1.4	1.2±1.3	<0.0009
Henneguya spp.[b]	G,M,GB	0.7±1.1	0.3±1.5	0.4±0.7	1.0±1.4	<0.0009
Dermocystidium percae	F	1.1±4.8	17.6±81.1	17.6±77.6	5.4±40.8	
Tylodelphys clavata l	VB	44.5±44.2	0.3±1.5	25.2±32.0	14.2±24.0	<0.0009
Diplostomum gasterostei l	VB	10.0±10.0	0.5±0.8	3.5±4.7	8.1±9.9	<0.0009
Ichthyocotylurus variegatus l	C	4.3±23.2	1.0±2.6	8.9±12.8	1.9±3.4	
Bunodera luciopercae	I	27.7±45.2	6.3±8.7	21.7±76.0	3.1±5.9	<0.0009
Proteocephalus percae	I	2.6±5.7	0.1±0.3	0.3±0.8	0.4±1.4	<0.0009
Camallanus lacustris	I	0.2±1.1	1.0±1.6	6.0±9.0	0.8±1.8	<0.0009
Acanthocephalus lucii	I	2.9±5.1	2.0±3.1	0.9±2.1	1.4±3.4	<0.0009
Glochidia l[c]	G,F	—	0.6±1.2	6.7±11.5	19.0±65.7	

Note: Values are given as the mean ± SD. Only species attaining a level of 1.0 in at least one lake are included. Sample sizes for lakes Peurenka, Vatia, Saravesi, and Leppävesi were 123, 93, 119, and 120, respectively, for roach, and 118, 61, 120, and 110, respectively, for perch. Abbreviations are as in Table 2.

[a]Bonferroni-corrected.

[b]Numbers of myxosporeans determined on a 0–3 index; see text.

[c]Numbers of gill parasites determined on one side only; see text.

Table 5. Measures of component communities and infracommunities of parasites in roach and perch from four lakes in central Finland, 1986.

	Component community			Infracommunities			
	No. of fish	No. of taxa	1/SI	No. of fish	Taxa/fish	Parasites/fish	1/SI
Roach							
Lake Peurunka	123	29	11.4	103	7.1±1.9*a*	110.3± 83.0*a*	2.9±0.9*a*
Lake Vatia	93	30	12.6	76	6.2±1.9*b*	33.1± 22.6*b*	2.8±1.3*a*
Lake Saravesi	119	36	14.4	93	8.3±1.9*c*	106.3± 99.6*a*	3.2±1.3*b*
Lake Leppävesi	120	36	15.6	70	9.5±2.0*d*	153.0±121.0*c*	3.4±1.3*b*
Perch							
Lake Peurunka	118	31	14.0	102	7.9±1.8*a*	98.3± 80.0*a*	3.1±1.3*a*
Lake Vatia	61	29	14.4	44	5.8±2.0*b*	38.4± 95.0*b*	3.2±1.5*a*
Lake Saravesi	102	36	16.1	102	8.1±2.2*a*	99.4±122.3*a*	3.3±1.4*a*
Lake Leppävesi	93	34	16.2	93	7.9±2.0*a*	64.4± 84.3*c*	3.8±1.8*b*

Note: Infracommunity values are given as the mean ± SD. Values followed by different letters are significantly different.

Component communities

In both roach and perch, parasite taxon lists for the four lakes overlapped extensively. Most of the absent taxa were rare in the other lakes; exceptions are noted below. Only a few parasite taxa were limited to a single lake (two in roach, six in perch); all were rare. Parasite richness in both roach and perch was higher in the eutrophic lakes Saravesi and Leppävesi than in the polluted Lake Vatia or the oligotrophic Lake Peurunka (Table 5). Qualitative similarities (Jaccard indices) among component communities were high (74–84 in roach, 62–79 in perch). The highest similarities were between communities in fish from lakes Saravesi and Leppävesi; the lowest were between those from lakes Peurunka and Vatia (Table 6). Qualitatively, the basic pattern is one of the absence of species in the more polluted or oligotrophic lakes.

The quantitative data showed essentially the same patterns. In both fish species, parasite communities in lakes Saravesi and Leppävesi had more common species (prevalence >50%; Tables 2 and 3), had the highest diversities (Table 5), and were the most similar (Table 6). Quantitative similarities were high (65–86 in roach, 59–81 in perch), with the lowest similarity between parasite communities in lakes Vatia and Peurunka. All diversities were high, with the lowest levels in communities in Lake Peurunka.

The clearest patterns in species distributions are the higher prevalences of most digeneans; the absence of *Rhipidocotyle*

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Table 6. Similarity matrices for the parasite component communities in roach and perch in four lakes in central Finland, 1986.

	Peurunka	Vatia	Saravesi	Leppävesi
Roach				
Lake Peurunka	—	65	69	69
Lake Vatia	74	—	74	69
Lake Saravesi	76	78	—	86
Lake Leppävesi	81	83	84	—
Perch				
Lake Peurunka	—	59	72	75
Lake Vatia	62	—	69	66
Lake Saravesi	68	71	—	81
Lake Leppävesi	67	75	79	—

Note: Values above the diagonal are percent similarities, and the values below the diagonal are Jaccard similarities.

spp., *Sphaerostoma globiporum*, and glochidia larvae and the lower prevalences of nematodes in fish from Lake Peurunka (Figs. 2 and 3); the higher prevalences of *Dactylogyrus* spp. and *Dermocystidium percae* and the lower prevalences of digeneans (except for *Bunodera luciopercae*) and glochidia larvae in fish from Lake Vatia (Figs. 3 and 4); and the high prevalences of *Acanthocephalus lucii* in fish from both lakes Peurunka and Vatia (Figs. 2 and 4).

Infracommunities

Infracommunities varied markedly in all lakes and in both species of fish (Table 5). Nevertheless, roach in lakes Saravesi and Leppävesi had significantly more taxa per fish and higher diversities than those in the other two lakes, and those in Lake Leppävesi had significantly more parasites per fish than the others. Roach in Lake Vatia had significantly fewer taxa per fish and parasites per fish than those in the other lakes. Perch in Lake Leppävesi had significantly higher diversities of parasites, those in lakes Saravesi and Peurunka had significantly more parasites per fish, and those in Lake Vatia had significantly fewer taxa per fish and parasites per fish, than in the other lakes.

Two canonical discriminant functions, based on log-transformed intensity data for 398 roach or 341 perch from which all parasites were studied, differentiated fish from lakes Vatia, Peurunka, and a combination of lakes Saravesi and Leppävesi (Fig. 5). Fish from the last two lakes could not be separated satisfactorily. The percentages of correct classifications were 87, 90, and 93% in roach and 92, 96, and 76% in perch for lakes Peurunka, Vatia, and the pooled Saravesi–Leppävesi, respectively.

For the most part, the discriminating variables (Table 7) are those whose prevalences were significantly different in the four lakes. Seven variables were selected in roach. CV1 contrasts intensities of diplostomes versus those of *Rhipidocotyle fennica, Raphidascaris,* and glochidia and separates infracommunities in roach from the two eutrophic lakes from those in lakes Vatia or Peurunka. CV2 contrasts intensities of digeneans and glochidia versus *Raphidascaris* and dactylogyrids and separates infracommunities in roach from Lake Vatia from those in Lake Peurunka.

In perch, 12 variables were selected. CV1 contrasts intensities of those species with their highest prevalences and intensities in Lake Peurunka versus those less abundant in Lake Peurunka and separates infracommunities in perch from Lake Peurunka from those in the other three lakes. CV2 contrasts intensities of *Acanthocephalus lucii, Bunodera luciopercae,* and trichodinids versus those of glochidia, most other digeneans, *Trichodina urinaria,* and *Camallanus lacustris* and separates infracommunities in perch from Lake Vatia (and to a lesser extent, those from Lake Peurunka) from those in lakes Saravesi or Leppävesi.

Fig. 2. Prevalences (% of fish infected) of parasites that differ significantly (an asterisk indicates significance at Bonferroni-corrected level of 0.0025; a dash indicates nonsignificance) between Lake Peurunka (oligotrophic) and lakes Saravesi or Leppävesi (eutrophic). Only those parasites found in at least 15% of the fish in at least one lake are shown. Species more prevalent in Lake Peurunka are shown to the left and those more prevalent in at least one of the other lakes are shown on the right. Species codes: ACA LUC, *Acanthocephalus lucii*; ACH PER, *Achtheres percarum*; ALL ISO, *Allocreadium isosporum*; BUN LUC, *Bunodera luciopercae*; CAM LAC, *Camallanus lacustris*; DIP GAS, *Diplostomum gasterostei*; DIP SPA, *Diplostomum spathaceum*; GLO LAR, glochidia larvae; ICH MUL, *Ichthyophthirius multifiliis*; ICH VAR, *Ichthyocotylurus variegatus*; NEO JAP, *Neoergasilus japonicus*; PRO PER, *Proteocephalus percae*; RAP ACU, *Raphidascaris acus*; RHI CAM, *Rhipidocotyle campanula*; RHI FEN, *Rhipidocotyle fennica*; SPH GLO, *Sphaerostoma globiporum*; TRI NOD, *Triaenophorus nodulosus*; TYL CLA, *Tylodelphys clavata*; TYL POD, *Tylodelphys podicipina*; ZSC NOV, *Zschokkella nova*.

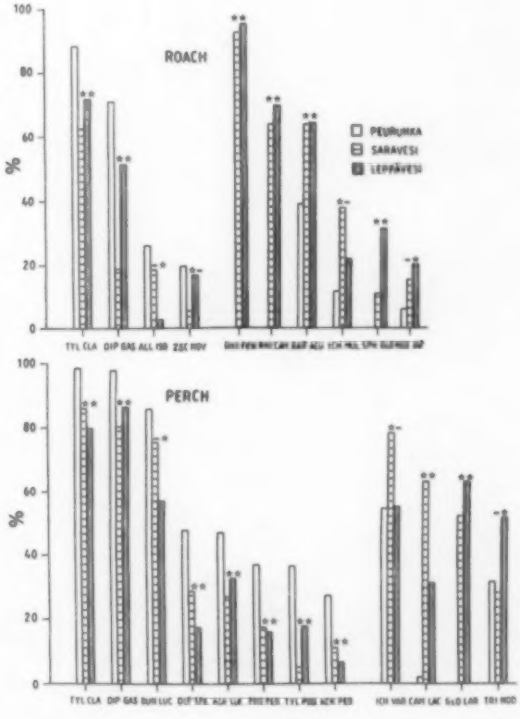

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Fig. 3. Prevalences of parasites that differ significantly (at Bonferroni-corrected level of 0.0025) between lakes Peurunka (oligotrophic and unpolluted) and Vatia (eutrophic and polluted). Only those found in at least 15% of the fish in at least one lake are shown. Species more prevalent in Lake Peurunka are shown to the left; those more prevalent in Lake Vatia are shown on the right. Species codes are as in Fig. 2. DAC SPP, *Dactylogyrus* spp.; DER PER, *Dermocystidium percae*.

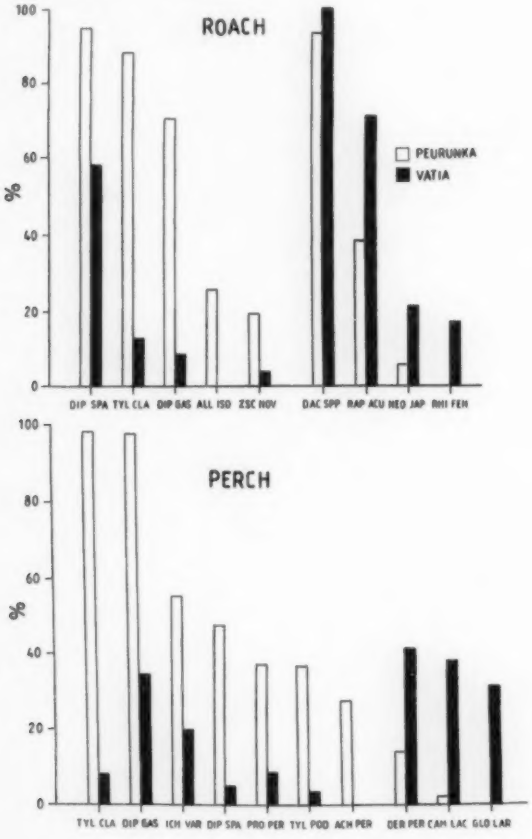
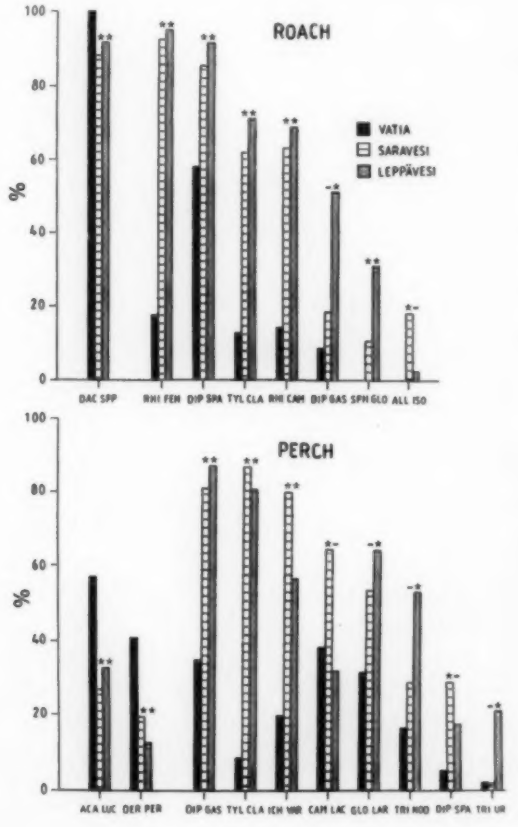

ROACH
PEURUNKA
VATIA
%
DIP SPA TYL CLA DIP GAS ALL ISO ZSC NOV DAC SPP RAP ACU NEO JAP RHI FEN
PERCH
TYL CLA DIP GAS ICH VAR DIP SPA PRO PER TYL POD ACH PER DER PER CAM LAC GLO LAR

Fig. 4. Prevalences of parasites that differ significantly (an asterisk indicates significance at Bonferroni-corrected level of 0.0025; a dash indicates nonsignificance) between Lake Vatia and lakes Saravesi or Leppävesi. Only those found in at least 15% of the fish in at least one lake are shown. Species more prevalent in Lake Vatia are shown to the left, and those more prevalent in at least one of the other lakes are shown on the right. Species codes are as in Figs. 2 and 3. TRI URI, *Trichodina urinaria*.


ROACH
VATIA
SARAVESI
LEPPÄVESI
%
DAC SPP RHI FEN DIP SPA TYL CLA RHI CAM DIP GAS SPH GLO ALL ISO
PERCH
ACA LUC DER PER DIP GAS TYL CLA ICH VAR CAM LAC GLO LAR TRI NOD DIP SPA TRI UR

1994 data

In 1994, following several years of reduced chemical pollution and nutrient loading, the index parasites in Lake Peurunka (unaffected by the changes) had prevalences indistinguishable from those of 1986 (except for reductions in the prevalences of *Raphidascaris acus* and *Diplostomum gasterostei*; Table 8). In Lake Vatia, however, prevalences of many parasites had changed significantly. Most of these changes (the increases in glochidia and *Rhipidocotyle* spp. in roach and *Diplostomum gasterostei, Ichthyocotylurus variegatus* and *Achtheres percarum* in perch) were in the direction anticipated from reduced pollution. However, the decrease in *Tylodelphys clavata* and the increase in *Raphidascaris acus* were not. In Lake Saravesi, two parasites, *Rhipidocotyle campanula* and *Diplostomum gasterostei*, increased significantly; both were in the direction expected from reduced pollution. Other species had not changed.

Discussion

We have assumed that the lakes we studied were initially very similar and that the differences among them are due primarily to the changes occurring over the past 50 years. These assumptions were based on the historical background and the nature of the fish fauna of northern Europe. They are supported by the wide distributions of the native species of fishes in Finland (Koli 1984) and by unpublished catch statistics, which show the same fish fauna in lakes both above and below our study area. In our data, the strong similarities among these four lakes, both in their fish faunas and in the parasites of roach and perch, and our inability to separate infracommunities of parasites in fish from lakes Saravesi and Leppävesi are consistent with the assumption that the fish (and parasite) faunas were originally very similar. The major difference in the fish faunas in 1986, the scarcity of coregonids from lakes Vatia and Saravesi, is that expected in polluted water bodies (Hakkari 1992). The

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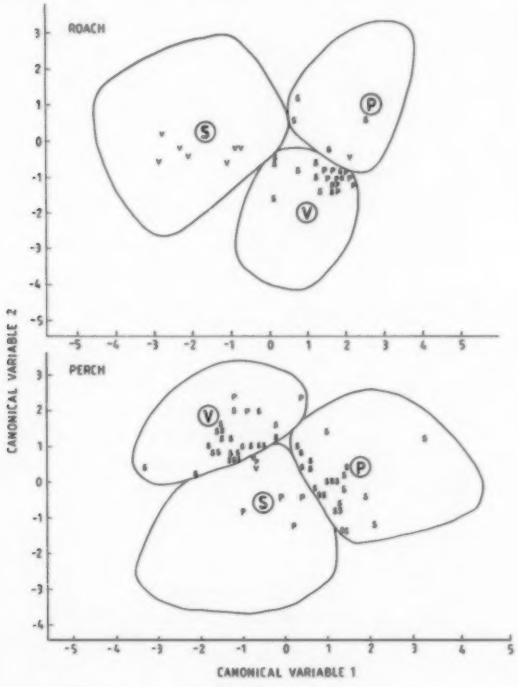
Fig. 5. Plots of locations on the first two canonical variables from a discriminant function analysis of parasite infracommunities in roach and perch from four lakes in central Finland. Outlined areas enclose locations classified by maximum likelihood as the pooled lakes Saravesi and Leppävesi, which could not be separated (abbreviated S), Lake Peurunka (P), or Lake Vatia (V). Group centroids are indicated by the large, circled letter. Misclassified infracommunites are indicated by a small letter indicating the true source. For clarity, data points for correctly classified infracommunities are not shown.



increase in the numbers of these species with decreased pollution in the 1990s adds further support to our assumption that the differences in the fish faunas in 1986 were environmentally induced.

Of course, similarity in fish faunas does not necessarily imply similarity in parasite faunas (Kennedy 1978; Hartvigsen and Kennedy 1993). However, most studies comparing parasite faunas have investigated isolated lakes, not those with extensive interconnections. To our knowledge, the only study involving interconnected lakes in the same watershed is that of R. Hartvigsen and O. Halvorsen (unpublished data), who found that samples taken from lakes in the same chain were as similar as samples taken from different parts of a single lake. We do not expect that the parasite faunas in our lakes were identical prior to anthropogenic changes, merely that they were similar enough so that infracommunities could not be separated satisfactorily, just as in our material from lakes Saravesi and Leppävesi.

Our assumption that Lake Peurunka had a fish (and parasite) fauna very similar to those of the other lakes is less secure. It is connected to Lake Vatia by a much smaller stream, with a greater elevational gradient. It has no large lake upstream

Table 7. Discriminating parasites and their coefficients for the first two canonical discriminant variables (CV1, CV2) that separate roach or perch from four lakes in central Finland.

	F^a	CV1	CV2
Roach			
Rhipidocotyle fennica	570.40	−0.977	0.250
Diplostomum spathaceum	130.16	0.248	0.584
Raphidascaris acus	22.21	−0.164	−0.481
Tylodelphys clavata	22.64	0.052	0.429
Diplostomum gasterostei	11.21	0.223	0.241
Glochidia	9.84	−0.156	0.234
Dactylogyrus spp.	7.95	0.085	−0.268
Perch			
Tylodelphys clavata	81.76	0.619	−0.571
Glochidia	51.36	−0.118	−0.717
Camallanus lacustris	42.70	−0.492	−0.236
Proteocephalus percae	21.27	0.407	0.045
Diplostomum gasterostei	16.82	0.317	−0.228
Achtheres percarum	13.53	0.366	0.042
Acanthocephalus lucii	9.39	−0.038	0.323
Bunodera luciopercae	9.16	0.131	0.319
Diplostomum spathaceum	6.43	0.280	−0.004
Trichodina spp.	5.06	0.233	0.154
Ichthyocotylurus variegatus	5.89	−0.122	−0.309
Trichodina urinaria	5.30	0.071	−0.280

Note: Variables are listed in order of addition by the stepwise analysis run on log-transformed intensity data.

[a]Variance ratio for the addition of the variable to the discriminant function.

and has a much longer water retention time. These are all characteristics that could influence fish, invertebrate, and parasite faunas. In particular, we cannot be certain that the absence of *Anodonta* spp., and therefore species of *Rhipidocotyle*, did not precede the anthropogenic factors we are evaluating. However, the fish fauna, and the abundance of trematode metacercariae, *Achtheres percarum* and *Proteocephalus percae*, are similar to those of other oligotrophic lakes in Finland (discussed below).

The differences characteristic of Lake Vatia are most likely due to the effects of the more extensive chemical pollution there. The most characteristic pattern, the lower prevalences and abundances of digeneans, clearly reflected the pollution-caused paucity of molluscs (obligate first intermediate hosts) in Lake Vatia (Hynynen 1987). The exception, *Bunodera luciopercae*, which had a relatively high prevalence but relatively low abundance in Lake Vatia (compared with the other lakes), uses sphaerid clams, which were still present in Lake Vatia, as first intermediate hosts. Similarly, the low prevalence and abundance of glochidia in Lake Vatia reflected the paucity of *Anodonta piscinalis* and the absence of other unionids (Hynynen 1987). By 1994, following several years of reduced organochlorine input, the glochidia, both digeneans using unionids as first intermediate host and some of the other digenean metacercariae, had recovered to levels near those of Lake Leppävesi, providing further evidence that these changes were caused by pollution. Note, however, that the prevalence of *Tylodelphys clavata* in roach had decreased.

Three taxa showed significant increases in prevalence or abundance in Lake Vatia: dactylogyrids in roach and *Dermocystidium percae* and *Acanthocephalus lucii* in perch. *Asellus*

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Table 8. Prevalences of monitored index parasite taxa in roach and perch from three lakes in central Finland in 1986 and 1994.

	Lake Peurunka		Lake Vatia		Lake Saravesi	
	1986	1994	1986	1994	1986	1994
Roach						
Rhipidocotyle fennica	0	0	20	99[a]	92	91
Rhipidocotyle campanula	0	0	15	100[a]	53	99[a]
Diplostomum spathaceum	95	94	58	57	86	83
Diplostomum gasterostei	71	53[a]	9	12	19	38[a]
Tylodelphys clavata	88	77	13	2[a]	62	50
Raphidascaris acus	39	11[a]	72	88[a]	64	67
Glochidia	0	0	3	31[a]	12	23
Perch						
Dermocystidium percae	14	16	41	25	19	9
Diplostomum spathaceum	48	54	5	7	29	15
Diplostomum gasterostei	98	100	34	60[a]	80	83
Tylodelphys clavata	98	94	8	17	86	84
Ichthyocotylurus variegatus	55	65	20	61[a]	79	71
Achtheres percarum	27	33	0	10[a]	11	11
Glochidia	0	0	31	50	53	43

[a]Significantly different from the 1986 value, Bonferroni-corrected $p < 0.007$.

aquaticus, the intermediate host of *Acanthocephalus lucii*, is noted for its ability to prosper in stressed conditions (Murphy and Learner 1982). None was found in monitoring surveys of the benthic fauna of Lake Vatia (Hynynen and Meriläinen 1987; Meriläinen 1987), but *Asellus aquaticus* is most abundant in shallow littoral zones, rather than the deeper zones sampled in the monitoring studies. It is likely, but not proven, that the higher prevalence of this acanthocephalan is due to an increase in its intermediate hosts. Note, however, that this acanthocephalan was also more prevalent in Lake Peurunka than in lakes Saravesi or Leppävesi.

Immune responses have been considered important in regulating populations of various monogeneans, including species of *Dactylogyrus* (see review in Williams and Jones 1994). Immune responses of fish in Lake Vatia are impaired (Jokinen et al. 1995), and dactylogyrid populations increased rapidly on roach transferred from Lake Peurunka to a cage in Lake Vatia, but not in roach retained in a cage in Lake Peurunka (Bagge and Valtonen 1996). It seems likely that the increases in dactylogyrids, and in *Dermocystidium percae*, in Lake Vatia were due to the effects of chemical pollution on the immune system. Our results appear to differ from those of Thulin et al. (1988), who found that *Dactylogyrus* spp. on roach increased with distance from two pulp mills, one of which used chlorine bleaching. However, Thulin et al.'s sampling sites were very close to the mill (maximum distance 5 km), and fish were probably exposed to higher concentrations of pollutants. When parasitized roach were experimentally transferred to water containing higher concentrations of raw effluent, the numbers of dactylogyrids decreased significantly (R. Siddall, M. Koskivaara, and E.T. Valtonen, unpublished data).

Anikieva (1982) found that perch in an area of a lake affected by pulp mill effluents lacked the copepod *Achtheres percarum*, whereas the copepod was present in perch taken from unaffected areas of the same lake. In addition, Thulin (1983) found that the numbers of *Achtheres percarum* increased in samples as the distance from a pulp mill increased. *Achtheres percarum* was absent from Lake Vatia in 1986 but reached levels similar to those in Lake Saravesi in 1994. It seems likely that this ectoparasite may have been affected directly by the toxins in the effluent, as suggested by Khan and Thulin (1991).

Although the parasite communities in lakes Saravesi and Leppävesi were similar, there were a few taxa that differed significantly in prevalence between the two lakes. All three of those that were less prevalent in Lake Saravesi (*Diplostomum gasterostei* and *Sphaerostoma globiporum* in roach and *Trichodina urinaria* in perch) were also significantly less prevalent in Lake Vatia. Northern Lake Saravesi is close to, and receives water directly from, Lake Vatia; these species may have been influenced by chemical pollution. Note that the prevalence of *Diplostomum gasterostei* (the only one of the three that was studied in 1994) in roach from Lake Saravesi did increase with the reduction in pollution between 1986 and 1994.

The differences characteristic of Lake Peurunka appear to reflect a complex of factors. The absence of *Rhipidocotyle* spp. and glochidia is clearly due to the absence of unionid clams, substantiated by diving surveys (J. Taskinen, University of Jyväskylä, Jyväskylä, Finland, personal communication). Such clams are common in virtually all lakes in central Finland, including most oligotrophic lakes (J. Jokela, University of Turku, Turku, Finland, personal communication); glochidia were present in all but 2 (Lake Peurunka and one other) of 22 lakes (or sites) in the Päijänne drainage system (E.T. Valtonen, unpublished data). Given the oligotrophic nature of this lake, it seems likely that populations of these clams were low, and became locally extinct, possibly owing to predation by muskrats (*Ondatra zibethica*), which can effectively eliminate clams over extensive areas within lakes (Jokela and Mutikainen 1995). The dam now prevents any rescue by migration of glochidia-infected fish from Lake Vatia. This may be an example of the influence of fragmentation.

It also seems likely that some of the differences reflect the lack of pollution or eutrophication; the two cannot be distinguished in our data. *Achtheres percarum* in perch was absent in Lake Vatia and significantly more prevalent in Lake Peurunka than in lakes Saravesi and Leppävesi. In addition,

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although *Achtheres percarum* recovered in Lake Vatia with reduced pollution and reached the same prevalence as in perch from Lake Saravesi, both were significantly less than that in perch from Lake Peurunka.

The significantly higher prevalences of most adult or metacercarial digeneans in Lake Peurunka also may be related to its unpolluted condition. Wisniewski (1958) and Esch (1971) suggested that eutrophic lakes would be dominated by metacercaria of species maturing in fish-eating birds or mammals, whereas oligotrophic lakes would be dominated by species maturing in fish. That is not the case with our data, nor is it characteristic of other oligotrophic, unpolluted water bodies in northern Finland, where metacercaria of species maturing in fish-eating birds are at least as common as in Lake Peurunka. More than 90% of roach and 90% of perch of oligotrophic and oligohaline Bothnian Bay and 88% of perch of oligotrophic Lake Yli-Kitka in northeastern Finland had some digenean metacercariae in their eyes (E.T. Valtonen and D.I. Gibson, unpublished data). The high prevalences of both adult digeneans and strigeid metacercariae in Lake Peurunka appear to reflect the normal condition in unpolluted Finnish water bodies, including oligotrophic ones. This conclusion also applies to *Proteocephalus percae*, which is very common in such lakes (Valtonen and Rintamäki 1989).

The differences between parasites from Lake Peurunka and those from lakes Saravesi or Leppävesi, therefore, probably reflect all three factors (fragmentation, pollution, and eutrophication), but the influences of eutrophication are not separable from those of pollution. This is not unexpected in a system in which eutrophication and chemical pollution stem from the same point source.

This system does provide several insights. First, by preventing a rescue by parasites carried by migrating fish, fragmentation has allowed the differentiation of the parasite community in at least one lake. This case involves the disappearance of a clam with an obligate parasitic larval stage in the fins and gills of fish and of the parasites developing in that clam. However, it should be noted that the parasite faunas of lakes Saravesi and Leppävesi have not diverged significantly with about 50 years of isolation.

Second, it shows that some of the effects of pollution can be effectively reversed in a relatively short time, provided that remnant populations, or a source of colonists, are available. This system appears to be an excellent one in which to track rehabilitation effects, and a study to do that is in progress.

Third, it follows Janovy et al. (1992) and Kennedy (1993) in showing that analyses based on infracommunities can be considerably more powerful than those based merely on summed data (the species lists, their prevalences, and abundances). The discriminant analysis clearly showed that there were two major axes in the differences between communities (and identified the parasite taxa associated with each), whereas the patterns of species presence and abundances did not. The discriminant analysis also showed clearly that, despite significant differences in the prevalences of some parasite species, the parasite faunas in lakes Saravesi and Leppävesi could not be separated.

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The influence of temperature and food chain length on plankton predator–prey dynamics

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Abstract: With recent predictions for global climate warming, the question arises as to how changes in temperature influence the dynamics of populations in natural communities. We investigated the effect of temperature (18 and 25°C) on the stability of a common freshwater predator–prey system consisting of *Daphnia pulex* and phytoplankton in different types of mesocosm communities. The plant–herbivore dynamics were examined in the presence and absence of the predator *Mesostoma ehrenbergii* to simulate food chains of two or three trophic levels, and in the presence or absence of competing zooplankton. The predator–prey system was destabilized at the higher temperature in all types of communities, and *D. pulex* extinction always occurred. This result was unaffected by simplification of the community but was enhanced by the addition of the carnivore. Mechanisms are proposed for the observed results including (*i*) the direct effect of temperature on vital rate parameters describing the *D. pulex* – algal interaction and (*ii*) a shift in algal community structure to less edible species following suppression by the herbivore at the higher temperature. Some implications of temperature increases expected under current global warming scenarios in pond systems are discussed.

Résumé : Dans le contexte de la prédiction d'un réchauffement climatique global, on peut se demander comment les changements thermiques vont influer sur la dynamique des populations dans les communautés naturelles. Nous avons étudié l'effet de la température (18 et 25°C) sur la stabilité d'un système prédateur–proie courant en eau douce, constitué de *Daphnia pulex* et de phytoplancton, dans différents types de communautés au niveau du mésocosme. La dynamique végétaux–herbivore a été examinée en présence et en l'absence du prédateur *Mesostoma ehrenbergii* pour simuler les chaînes alimentaires de deux ou trois niveaux trophiques, et en présence ou en l'absence de zooplancton concurrent. Le système prédateur–proie a été déstabilisé à la température élevée dans tous les types de communautés, et on a toujours observé l'extinction de *D. pulex*. Ce résultat n'a pas été touché par une simplification de la communauté, mais a été accentué par l'ajout du carnivore. Nous proposons des mécanismes expliquant les résultats observés, notamment (*i*) l'effet direct de la température sur les paramètres vitaux qui décrivent l'interaction entre *D. pulex* et les algues, et (*ii*) une évolution de la structure de la communauté algale vers des espèces moins comestibles après l'élimination due à l'herbivore à la température la plus élevée. Nous analysons certaines incidences des augmentations de température prévisibles dans les systèmes lentiques selon les scénarios actuels de réchauffement planétaire.
[Traduit par la Rédaction]

Introduction

Although temperature has an overriding effect on rates of biological processes, there are few studies that examine how changes in temperature affect the dynamics of interacting populations. In plankton systems, there have been two benchmark population dynamic studies regarding the effect of temperature (Pratt 1943; Halbach 1970). Pratt (1943) raised laboratory populations of *Daphnia magna* at 18 and 25°C and noted a switch from stable population dynamics at 18°C to wildly fluctuating dynamics at 25°C. Halbach (1970) similarly noted that for rotifer (*Brachionus calyciflorus*) populations, the dynamics were destabilized with an increase in temperature. Changes in dynamics observed in both studies have been attributed to the effect of temperature on physiological rates that determine the population growth rates and the time lags of the zooplankton population (May 1981; Murdoch and McCauley 1985; Lawton 1987; McCauley and Murdoch 1987). At the individual level, temperature affects phytoplankton growth rates (DiToro et al. 1975; Ahlgren 1987; Hanagata et al. 1992) and metabolic, filtering, and assimilation rates of zooplankton (Obreshkove and Abramowitz 1932; Hall 1964; Lampert 1977*a*; Vijverberg 1980). These physiological and growth rate changes might be expected to be reflected in population parameters for the *Daphnia*–algal interaction. Both Pratt (1943) and Halbach (1970) conducted population studies on single species of herbivores where prey populations were nondynamic (i.e., prey were added at intervals during the experiment). The influence of temperature on the stability of a predator–prey system in which both prey and predators are dynamic remains to be determined.

Investigating the effects of changes in temperature on population dynamics is increasingly important given the anticipated effects of global warming on aquatic systems. Shallow water bodies common in the North American prairies are among the first ecosystems expected to show the effects of global warming, since in these systems, water temperature closely mimics ambient air temperature (Carpenter et al. 1992). In the prairie regions, summer increases of up to 9°C are expected with

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doubling of atmospheric CO_2 (Schindler et al. 1990). Increasing ambient temperature has the potential to alter the physiological rates of individuals, the vital rate parameters describing populations, and their resultant population dynamics (May 1981; Murdoch and McCauley 1985; Lawton 1987; McCauley and Murdoch 1987).

Plankton systems are useful for answering questions regarding population dynamics for several reasons. Among zooplankton, cladocerans are distributed ubiquitously and often represent important herbivores and carnivores in freshwater habitats (Mourelatos and Lacroix 1990). *Daphnia* spp. are typically the dominant cladocerans in lentic systems (Goss 1978; Hebert 1978), and they are ideal for the study of population dynamics, having relatively short generation times and well-documented ecology (i.e., physiological ecology, patterns of predation, and competition) (e.g., Peters and De Bernardi 1987; McCauley et al. 1990*b*). Furthermore, the dynamics of mesocosm and field *Daphnia* populations are qualitatively similar (McCauley and Murdoch 1987; McCauley and Murdoch 1990; Leibold and Wilbur 1992).

The purpose of this study is to examine the effect of increasing temperature within a sublethal range on the dynamics of a plant–herbivore interaction within mesocosm systems. To determine how the response to temperature is altered by biotic interactions, we compare and contrast the effects of temperature in several community types. We investigate plant–herbivore dynamics in food chains of two lengths (two and three trophic levels) embedded within communities at two levels of complexity (baseline and simplified food webs) in an unbalanced factorial design. Our food chains were composed of phytoplankton and *Daphnia pulex* with the addition of the flatworm carnivore *Mesostoma ehrenbergii* in the three-level food chains. The comparison of phytoplankton – *D. pulex* dynamics in diverse and simplified communities allows for the determination of how the presence of an initially more complex phytoplankton assemblage and competitors of *D. pulex* can alter the dynamic response to temperature. Beisner et al. (1996) examined the effects of *M. ehrenbergii* on the dynamics of *D. pulex* at different temperatures. In this paper, we consider these results and contrast them with the temperature effects in two-level systems.

Materials and methods

Plankton communities were maintained in 650-L rectangular polyethylene tanks housed under constant conditions in the EFFECT facility at the National Hydrology Research Institute in Saskatoon, Saskatchewan. Pond water was obtained from a local pond (with a measured total phosphorus concentration of 40 µg/L) and was added to all tanks on May 17, 1993 (day 137 of the year). Eighteen tank communities were reared at two temperatures (nine at each temperature): 18 and 25°C. A 7°C temperature difference was chosen to represent a likely mean temperature change under predicted global warming scenarios (Schindler et al. 1990). In addition, the two temperatures were chosen to ensure that both were nonlethal for the species used in the experiment: the flatworm carnivore *M. ehrenbergii* (Heitkamp 1977) and a warm-temperature clone of *D. pulex* (McCauley and Murdoch 1987; E. McCauley, unpublished data). The 18°C water temperature was maintained by keeping the ambient air temperature at 18°C in the EFFECT chamber. Aquarium immersion heaters were used for the 25°C treatments. Inoculated *D. pulex* and *M. ehrenbergii* were acclimated to the tank water temperatures by addition to flasks containing 1 L of pond water at room temperature. Flasks were then suspended in the tanks for 24 h prior to release.

To investigate the influence of community structure (i.e., presence of potential competitors and predators) on the temperature response of the *D. pulex* – algal interaction, several mesocosm communities were set up and are described below: baseline communities (two trophic levels), simplified communities (two trophic levels), and systems with three trophic levels (embedded in the baseline community type). Note that Beisner et al. (1996) presented part of the data from the baseline communities with two and three trophic levels in investigating temperature-mediated carnivore–herbivore dynamics. They did not consider the *D. pulex* demographic data for these treatments nor the entire data set for simplified communities. The present paper concerns with the effect of temperature on plant–herbivore dynamics in various community types.

Baseline communities (phytoplankton + *D. pulex* and competitive grazers)

The effect of temperature on *D. pulex* – algal population dynamics was investigated in a baseline community type. The baseline communities consisted of pond water that was filtered through 60-µm mesh as it passed into the tanks. Filtration to this level allowed for communities of phytoplankton, protozoans, rotifers, and eggs of other crustaceans to be introduced into the tanks but excluded any potential invertebrate predators of *D. pulex* such as species of *Chaoborus* or *Notonecta*. There was a total of six baseline tanks with three replicate tanks at 18°C and three at 25°C. Following an identical schedule for all tanks, 65 individuals from a single clone of *D. pulex* were then added to each tank over a period of 14 days (days 152–166 of the year) and the mid-date of this period (day 159 of the year) was taken as day 0 of the experimental period. These baseline communities thus consisted of two trophic level food chains of *D. pulex* (inoculated into the system) and their phytoplankton prey (from the pond water) within a community of herbivores and algae. Examination of preserved samples showed that heterotrophic flagellates, rotifers, *Alona* sp., ostracods, and *Ceriodaphnia* sp. were all present at low biomass levels in these communities (see Results).

Simplified communities (phytoplankton + *D. pulex*)

To test the hypothesis that *D. pulex* – algal dynamics can be altered by the presence of herbivore competitors and the initial complexity of the algal species assemblage, simpler communities were also set up in six tanks (three replicates at each temperature) by further filtering the pond water through three successive 20-µm screens. By this process, potential competitors such as other cladocerans were removed, the initial densities of rotifers were reduced, and the initial phytoplankton species composition was simplified, as verified by counts of preserved samples. *Daphnia pulex* individuals were inoculated into these tanks as described for the baseline community type.

Communities with three trophic levels (phytoplankton + *D. pulex* and competitive grazers + carnivore)

Systems with three trophic levels were also created by inoculating tanks with the carnivore *M. ehrenbergii* (Turbellaria). In these experiments, the dynamics of the *M. ehrenbergii* population itself are not of direct interest and therefore are reported elsewhere (Beisner et al. 1996). Only the influence of the carnivore's feeding and population growth on the *D. pulex* – algal response to temperature is of concern. *Mesostoma ehrenbergii* can respond numerically to prey density within a single growing season because they reproduce viviparously via the production of subitaneous eggs (Heitkamp 1977). This carnivore is a common flatworm predator of *D. pulex* in northern temperate ponds (Göltenboth and Heitkamp 1977) and possesses a generation time close to that of *D. pulex* (Heitkamp 1977). Three replicate tanks at each temperature, containing the same communities as in the baseline tanks, were inoculated with the carnivore species. *Mesostoma ehrenbergii* were collected from a pond northwest of Calgary,

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Alberta. Following establishment of *D. pulex* populations, from day 169 to day 177, a total of 110 juveniles and 40 subitaneous adult carnivores were added to each of the six, three-level treatment tanks (three at 18°C and three at 25°C). This density of *M. ehrenbergii* (230 individuals/m^3) is comparable with lower summer levels of *M. ehrenbergii* in natural ponds (Heitkamp 1972).

Sampling methodology

All 18 tanks received a constant air supply using an airstone. Water temperature in each tank was monitored on every sampling date along with water conductivity and pH. Water temperature remained within ±1°C of the treatment value throughout the experimental period; no marked changes in pH or conductivity were observed and there were no differences between treatments. Tanks were mixed twice weekly (at sampling time), and there was no evidence of thermal stratification. Each tank was individually illuminated on a 12 h light : 12 h dark regime by a bank of four Sylvania wide-spectrum Gro-Lux bulbs positioned 1 m above the surface of the water. To replace water lost to evaporation, membrane-filtered, autoclaved pond water and distilled water were added to all tanks, as required. Constant conditions were maintained throughout the entire experimental period (150 days) from June to early November 1993 (days 159–309). Twice weekly, samples were taken for phytoplankton, rotifers, and crustacean zooplankton. Before sampling, tanks were well mixed, and following sampling, all filtered water was returned to the source tank.

Phytoplankton biomass was estimated primarily using chlorophyll *a* (chl *a*) analysis. A 1-L whole water sample was taken from the middle of each tank. Chlorophyll *a* concentration was determined using ethanol and a fluorometer (Nusch 1980) for size-fractionated subsamples (<35 μm). A phytoplankton sample preserved with Lugol's iodine (Lund et al. 1958) was also taken for later identification and enumeration. This preserved sample was a subsample of 3 L of water drawn from three random locations in the tanks. Only selected Lugol's samples were actually examined; samples were enumerated for periods in which rapid changes in phytoplankton biomass (as estimated from chl *a* concentration) occurred, to determine if taxonomic shifts were also occurring. This meant choosing a sample from the time when phytoplankton biomass was increasing late in the experiment (during the large *D. pulex* decline) and contrasting it with a sample taken early in the experimental period (during the peak *D. pulex* biomass period) (see Results, Table 1). For enumeration, a 10-mL subsample was settled and enumerated using an inverted microscope. A minimum of 400 individuals in the most common taxa were counted to reduce counting error to within 10% (Lund et al. 1958). Larger phytoplankton were measured and identified to species, while smaller phytoplankton (e.g., <35 μm) were classified on the basis of size and the possession of flagella. Biomass was estimated from biovolume on the basis of the size and shapes of phytoplankton.

For zooplankton, four 1-L samples were drawn from different areas of each tank. Samples were combined and filtered through a 35-μm mesh net, and zooplankton were anaesthetized with carbonated water and preserved in a 4% sugar–formalin solution. Samples were completely censused and the major taxonomic groups of zooplankton identified using a dissecting microscope. The density of *D. pulex* was recorded for five size-classes (S1, ≤0.8 mm; S2, 0.8–1.0 mm; S3, 1.0–1.4 mm; S4, 1.4–2.0 mm; S5, ≥2.0 mm) and clutch size was recorded for each fecund adult (cf. McCauley and Murdoch 1990). Densities were converted to biomass using the relationship $W = 0.006\,24\,L^{2.4}$, where L is the body length (millimetres) at the midpoint for each size-class and W is the dry weight (milligrams) (E. McCauley, unpublished data). Rotifers were identified to genus and counted with an inverted microscope by enumerating the contents of a settled 10-mL subsample.

Statistical analyses

Temporal dynamics for the phytoplankton biomass (as estimated by chl *a* concentration, >35 μm), total *D. pulex* biomass, and *D. pulex* demography were examined. For the *D. pulex* demographic data, individuals less than 1 mm (S1 + S2) were classified as juveniles, individuals between 1 and 1.4 mm (S3) were classified as adolescents, and individuals greater than 1.4 mm (S4 + S5) were mature and were classified as adults (McCauley and Murdoch 1990). Individuals less than 1.4 mm are virtually never reproductive, although it is possible for adults also to be nonreproductive, especially if food levels are low (McCauley et al. 1990*a*).

Owing to the short time series observed at 25°C because of extinctions (see below), a formal time-series analysis to test for temperature effects was not feasible. Statistical tests of our hypotheses involve analysis of variance (ANOVA) comparisons, and to avoid violating assumptions relating to autocorrelation, we used derived variables and response functions to characterize the time series. Although the use of these variables does not rid the data of autocorrelation, this method provides an effective remedial measure to account for time-dependent observations and permits the use of ANOVA (Mead 1988). For the *D. pulex* populations, response variables included the first maximum biomass level, the date this maximum occurred, the persistence time of the populations, and the population growth rate (response function). The first maximum biomass level was the first measured peak in the *D. pulex* biomass time series. The persistence time of the populations was measured as the time period between the start of the experiment (day of year 159, experimental day 0) and the first of two consecutive dates on which there were zero values for *D. pulex* biomass followed by no later significant increases in biomass (e.g., above 0.05 mg/L). Finally, the *D. pulex* population growth rate was measured as the slope of the log biomass versus time relationship calculated from the date of inoculation to the date for which the first maximum biomass level was recorded. For the phytoplankton time series, the response variable of interest was the minimum level of phytoplankton measured as the average of the phytoplankton biomass values on the date of the first maximum *D. pulex* biomass and the date preceding it.

Each response variable was examined in a fractional three-way factorial ANOVA (sensu Neter et al. 1990) with two levels of temperature (18 and 25°C), two levels of community complexity (baseline and simple), and two levels of food chain length (two and three). The experimental design was unbalanced because of the absence of a simplified community type with three trophic levels. Contrasts and Tukey's procedure were used to determine relationships between subgroups of factors. The data were log transformed to reduce heteroscedasticity.

Graphical representation of the data in this paper shows a period of time that is shorter than the entire experimental period of 150 days. This is to permit detailed examination of the dynamics and demography during the relevant time period for the populations at 25°C, which is approximately for the first 50 days of the experimental period. The major results from the entire experimental period are discussed in the text, however.

Results

Effect of temperature in baseline communities

In the baseline treatments, the *D. pulex* – algal interaction was destabilized by the increase in temperature from 18 to 25°C (Fig. 1). The persistence time of the *D. pulex* populations was significantly shortened at 25°C, being reduced to 77 ± 14.3 days (mean ± SE) from an average of 129 ± 21 days at 18°C, although there was an interaction with food chain length that will be discussed in a later section of the Results (effect of lengthening the food chain). The *D. pulex* populations always went extinct at 25°C, while all populations persisted over the same time period at 18°C (Fig. 1). In all cases at 18°C, the

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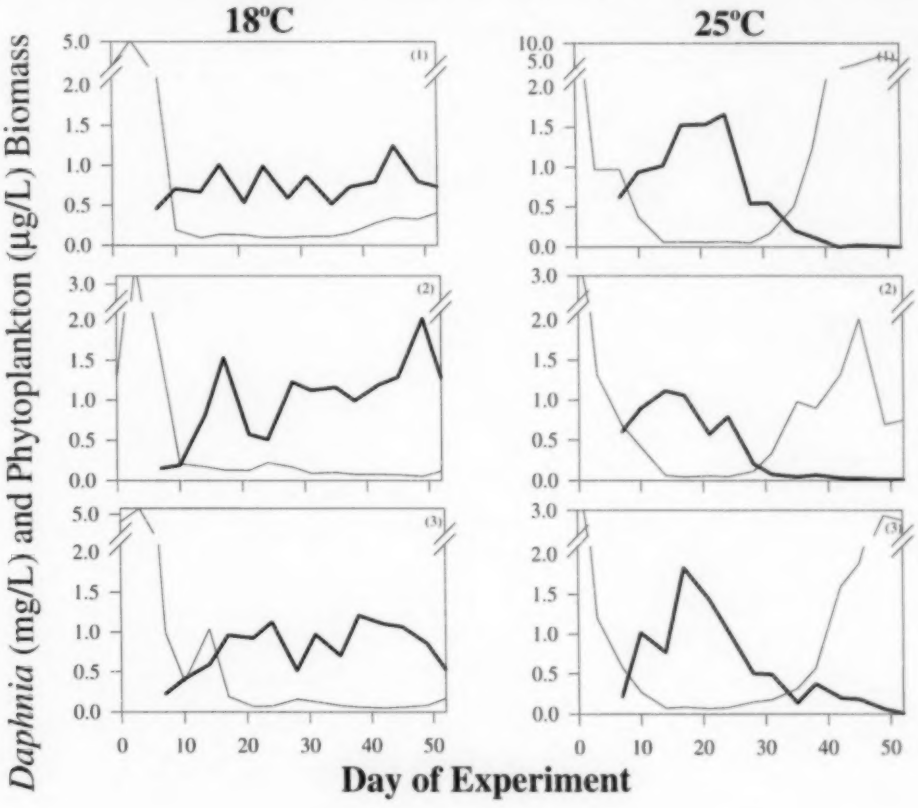
Fig. 1. Total *D. pulex* biomass (heavy lines) and phytoplankton biomass (as estimated by chl *a* concentration) (thin lines) for the three replicate tanks in the 18°C and 25°C baseline treatments (phytoplankton + *D. pulex* +competitive grazers).



D. pulex biomass declined slowly (after approximately two generations). In general this is an observation common to other long-term mesocosm experiments, although an explanation is lacking (S. Young, Imperial College at Silwood Park, Ascot, personal communication; W.E. Neill, Department of Zoology, University of British Columbia, Vancouver, B.C., personal communication). In one tank, replicate 3, the *D. pulex* population did not persist but declined slowly to extinction. As a result, the average persistence time for the 18°C treatment is shorter than the entire duration of the experimental period of 150 days. Because of the different manner in which this population extinction occurred, it is unlikely that it was a result of the same mechanism that gave rise to the rapid extinctions seen at 25°C.

The growth rates of *D. pulex* populations differed significantly with temperature (ANOVA, $P = 0.0211$). Growth rates for the baseline communities increased from 0.019 ± 0.006 day^{-1} at 18°C to 0.032 ± 0.001 day^{-1} at 25°C. In general, at both temperatures, phytoplankton were reduced to low levels by day 14, while *D. pulex* continued to increase to their maximum around day 19 (Fig. 1). This suggests that *D. pulex* were able to overshoot their carrying capacity (e.g., Goulden and Hornig 1980; McCauley and Murdoch 1987) and that this ability was unaffected by temperature. There was no significant effect of temperature on the first maximum in *D. pulex* biomass levels (1.22 ± 0.16 mg/L at 18°C and 1.54 ± 0.22 mg/L at 25°C; $P = 0.5012$) nor on the associated minimum level of edible phytoplankton biomass (0.09 ± 0.03 mg/L at 18°C and 0.06 ± 0.01 mg/L at 25°C; $P = 0.3679$).

Algal biomass increased rapidly following the decline in the herbivore population at 25°C (Fig. 1). The structure of the phytoplankton community was investigated during this increase phase by enumerating some of the preserved Lugol's samples. For each baseline community tank, samples from two dates were enumerated: a date early in the experiment during the period when *D. pulex* biomass was high and phytoplankton biomass low and a later date during which the *D. pulex* populations were declining and phytoplankton biomass was increasing (Table 1). Over time, in the baseline community types, there was an increase in the relative proportion of inedible phytoplankton biomass >35 µm (Table 1). Small flagellates such as *Rhodomonas minuta*, *Gymnodinium* sp., *Kephyrion sitta*, *Chrysochromulina parva*, *Chromulina* sp., and nonflagellates (i.e., *Chlorella* sp.) were replaced by larger blue-greens such as *Anabaena lemmermanni* var. *minor* and *Schizothrix calcicola*. This shift was more pronounced over time at 25°C (Table 1).

In the baseline communities, other zooplankton species were entirely absent from samples for the duration of *D. pulex* persistence. These species increased in density predominantly

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Fig. 2. *Daphnia pulex* density in the juvenile (thin lines), adolescent (●) and adult (■) size-classes for the three replicate tanks in the 18°C and 25°C baseline treatments (phytoplankton + *D. pulex* + competitive grazers).

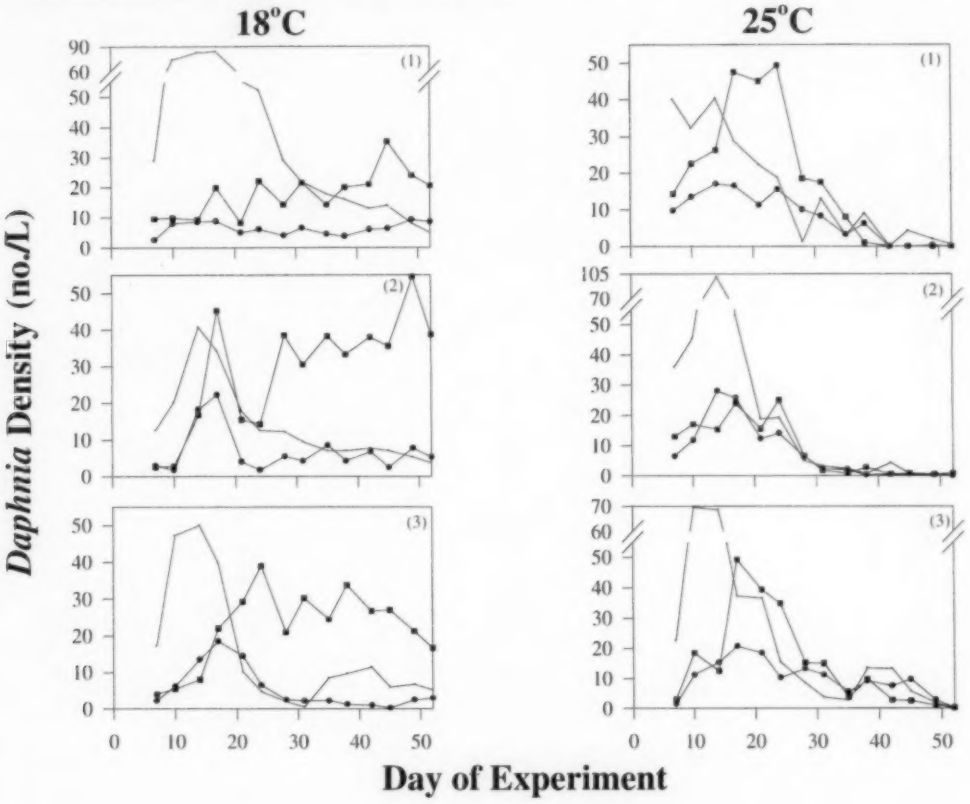

Table 1. The percent change per day in inedible phytoplankton biomass (>35 μm) for the counts done during the *Daphnia pulex* population peak and during the decline periods in the baseline community tanks.

	Replicate No.	Date of count during *D. pulex* peak (day of experiment)	Date of count during *D. pulex* decline (day of experiment)	% change/day in phytoplankton biomass >35 μm between two dates
18°C	1	28	87	–0.007
	2	31	56	+0.004
	3	35	66	+0.936
25°C	1	21	35	+0.357
	2	21	35	+3.143
	3	21	49	+2.857

at 25°C and only following the decline of *D. pulex*. Species of other zooplankton included *Alona* sp., *Ceriodaphnia* sp., rotifers, and ostracods. These data are not shown because other zooplankton species were not important to the overall *D. pulex* dynamics (Beisner 1994).

The demographic data for the *D. pulex* population showed that at 18°C, overlapping of size-classes occurred (Fig. 2). This was replaced by more cyclic behaviour, with one or two peaks in the density of all size-classes occurring at the higher temperature. Additionally at the higher temperature, extinction of the *D. pulex* populations occurred when cohorts of juveniles failed to mature to the adolescent and adult life stages (Fig. 2). There was no measurable effect of temperature on production of ephippia, which remained minimal throughout the experiment.

Effect of simplifying the community

No alteration in the qualitative or quantitative features of the *D. pulex* dynamics with temperature was noted by simplifying the food web (Figs. 1 and 3) despite the fact that the filtration method used to simplify the plankton community was effective. Statistically, there were no significant interactions between temperature and community type (i.e., simple versus baseline) on the response variables. Examination of samples showed that all larger zooplankton species present in the baseline community types (i.e., *Alona* sp., *Ceriodaphnia* sp., and ostracods) were excluded from the simplified systems throughout the experimental period. Extinction of *D. pulex* was again rapid as temperature was increased, while persistence for the duration of the experimental period of all populations occurred at 18°C. In two cases at 25°C, extinction of the *D. pulex* population occurred later than in any of the baseline community populations (replicates 1 and 2) (Figs. 1 and 3). No significant interaction with community simplification was noted, however, for the persistence times of the populations at 25°C. The

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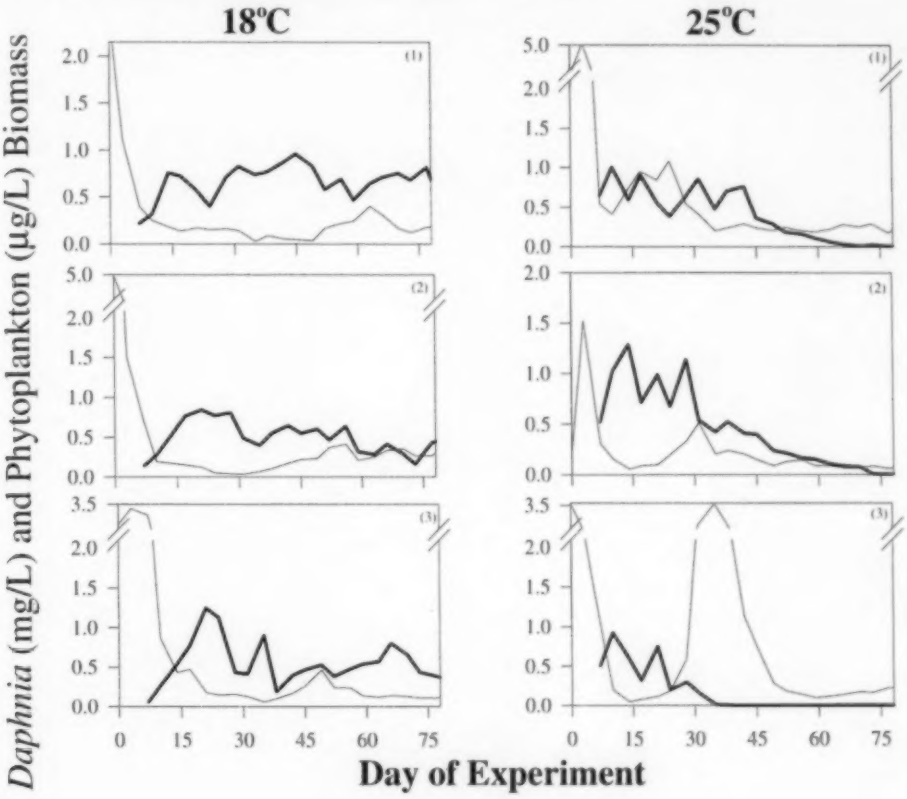
Fig. 3. Total *D. pulex* biomass (heavy lines) and phytoplankton biomass (as estimated by chl *a* concentration) (thin lines) for the three replicate tanks in the 18°C and 25°C simplified community treatments (phytoplankton +*D. pulex*).



effect of temperature was to reduce the persistence time from the entire experimental period (150 ± 0 days) at 18°C to 77 ± 19 days at 25°C. The major difference between the response of the simplified systems to temperature and that of the baseline community was that the proliferation of algae upon extinction of the *D. pulex* populations generally occurred to a much lesser degree in the former (except replicate 3) (Figs. 1 and 3).

Effect of lengthening the food chain

The addition of a third trophic level had an important effect on the response of *D. pulex* to temperature. In the presence of the carnivore, the effect of temperature was even more pronounced, with persistence of the *D. pulex* population being further reduced. There was an interaction effect of temperature and food chain length ($P_{interaction} = 0.0265$) (Fig. 4). The addition of a third trophic level to the predator–prey system at 18°C did not alter the persistence time of *D. pulex* (129 ± 21 days in two-level chains and 137 ± 13 days in three-level chains; $P = 0.6723$). At 25°C, the addition of the carnivore significantly reduced the herbivore persistence period from 77 ± 14 to 35 ± 0 days ($P = 0.0044$). There was no variation in the date of *D. pulex* extinction at 25°C in the presence of the predator. The algal community showed an increase in biomass concomitant with the decline in the *D. pulex* population and the *D. pulex* dynamics qualitatively resembled those in the baseline community (Fig. 5).

The *D. pulex* demographic data provided insight into the mechanism for the faster extinction of *D. pulex* in the presence of the carnivore at 25°C. The second, and in one case the third, pulses in juvenile (S1, S2, and S3) density observed in the baseline two-level tanks (Fig. 2) were absent from the three-level 25°C treatments (Fig. 6).

Discussion

The most important results in terms of *D. pulex* population stability were the unstable dynamics observed at the higher temperature, regardless of community structure. Across community types, at 18°C the populations generally persisted for the entire experimental period of 150 days (seven of nine replicates at 18°C). In the two cases (of nine total tanks) in which *D. pulex* extinction occurred at 18°C, extinction was much later than at 25°C and after a period equivalent to at least two generations of *D. pulex* at the temperature and low food level combination as estimated from Paloheimo and Taylor (1987). The populations were much less stable in all cases at 25°C where a single population fluctuation was observed, followed by extinction.

The *D. pulex* dynamics were similar in simplified and more diverse communities. Although the food webs differed in these two treatments (i.e., presence of grazing competitors and initial phytoplankton assemblage), there was no alteration of the

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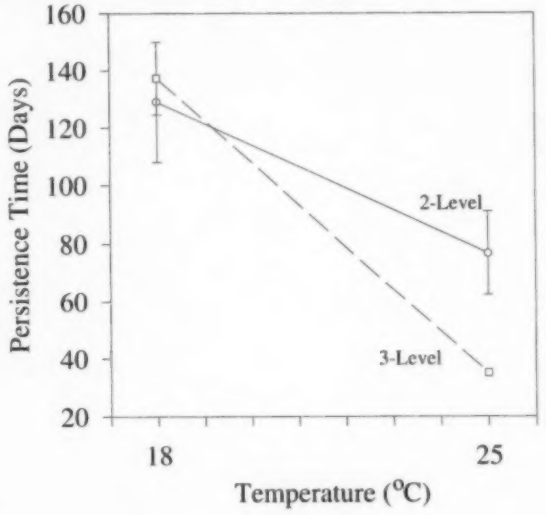
response to temperature. In both community types, *D. pulex* were able to regulate food resources despite differences in initial phytoplankton conditions.

The predator (*M. ehrenbergii*) enhanced the instability of the plant–herbivore interaction by further reducing persistence times of the *D. pulex* population at the higher temperature (for detailed results of the *M. ehrenbergii* – *D. pulex* interaction, see Beisner et al. (1996)). Generally, only one strong cohort of juvenile *D. pulex* was produced in the presence of the predator as opposed to two or three cohorts in the two-level systems. The results indicate that there is a much higher probability of destabilized *D. pulex* population dynamics at higher temperatures and that this effect is enhanced by lengthening food chains to three trophic levels.

We propose two mechanisms for the destabilization of *D. pulex* population dynamics with temperature in all community types. The first mechanism involves the direct effect of temperature on the rates of feeding, energy use, birth, and death for the *D. pulex* populations and time lags for the *D. pulex* – algal interaction such that the predator–prey equilibrium is destabilized. As predicted by theory, changes in these parameters can act to destabilize population dynamics by leading to larger amplitude fluctuations under structurally identical conditions (May 1981; Murdoch and McCauley 1985; Lawton 1987; McCauley and Murdoch 1987). Larger amplitude cycles increase the likelihood of extinction owing to stochastic processes, as the population trajectory approaches zero density more closely and more often.

Evidence for a direct destabilizing effect of temperature in our experiments is seen in the quantitative changes in the time series for the *D. pulex* and phytoplankton biomass with temperature. *Daphnia pulex* population growth rates were significantly faster at 25°C. In addition, *D. pulex* were able to quickly suppress phytoplankton biomass to very low levels at the higher temperature, and this may have led to extinction of *D. pulex* because of starvation under conditions where prey were dynamic. Although prey were suppressed to similar low levels at both temperatures, data from individual *D. pulex* (Lampert 1977*b*) indicate that *D. pulex* require proportionately more food at higher temperatures to meet the increased metabolic costs of higher temperatures. Thus, under dynamic conditions, both the levels of phytoplankton biomass and the relative turnover rates are important determinants of *D. pulex* survival (McCauley et al. 1988). Although elevated turnover rates would be expected for both phytoplankton (Hoogenhout and Amesz 1965) and *D. pulex* (e.g., Lampert 1977*b*; Goss 1978; Makarewicz and Likens 1979) with an increase in temperature, the extinction of the herbivore suggests that *D. pulex* grazing rates increased faster than the turnover rates of the phytoplankton so that starvation of the *D. pulex* population ensued. Characteristics of the *D. pulex* demography at the time of extinction further suggest that low phytoplankton levels were in part responsible for the herbivore extinction. At the higher temperature, extinction of the *D. pulex* population was preceded by an inability of juveniles to mature. It is likely that growing juveniles died upon reaching a threshold size at 25°C under conditions of increased metabolic requirements and low food levels. Small daphnids can survive at lower food densities than large ones, and there exists a threshold size above which no further growth can occur at low food densities (e.g., Kooijman 1986; McCauley et al. 1990*b*; Gurney et al. 1990). Our data show that extinction of *D. pulex* at 25°C was preceded by pulses in juveniles but by a failure of recruitment of these individuals to the mature and reproductive life stage. Dynamic instability owing to the effect of temperature on the vital rate parameters may therefore provide an explanation for the extinction of *D. pulex* populations at the higher temperature.

Fig. 4. Interaction diagram showing the *D. pulex* population persistence times at 18°C and 25°C for the baseline system with two trophic levels and the system with three trophic levels where the experimental period lasted 150 days. Error bars are ±1 SE. Note that there is no error associated with the persistence time of the populations in the three-level treatment at 25°C.



A second mechanism that can be proposed for the destabilization of *D. pulex* dynamics with temperature involves a change in algal community structure that is temperature dependent. This change from a highly edible to a less edible prey base leads to a shift in the *D. pulex* – algal equilibrium to a region of parameter space where this equilibrium is no longer feasible (e.g., Kretzschmar et al. 1993; Grover 1995). In a dynamic system, one would expect that with the decline of the *D. pulex* populations, a concomitant increase in phytoplankton would allow for a recovery of the *D. pulex* biomass. However, despite large increases in phytoplankton, the *D. pulex* populations were still unable to persist at 25°C. We suggest that this failure in the *D. pulex* population was caused by a disproportionate increase in inedible algae. We would expect the growth of inedible algae to be promoted under the dynamic conditions in our experiment for several reasons. First, from observations of temperate freshwater systems, zooplankton grazing can alter the dominance in the algal competitive arena over a growing season (Porter 1977; McCauley and Briand 1979), leading to a shift in the phytoplankton community from a highly edible to a less edible one (e.g., Schindler 1970; Arnold 1971; Lampert et al. 1986; Sterner 1989). Second, an increase in temperature is expected to elevate rates of nutrient supply to freshwater systems because of enhanced rates of microbial decomposition (Saunders 1980; Blumberg and DiToro 1990; Cooper 1990). In nutrient-rich systems, the growth of less edible species may be favoured over that of the more edible ones (Smith 1982;

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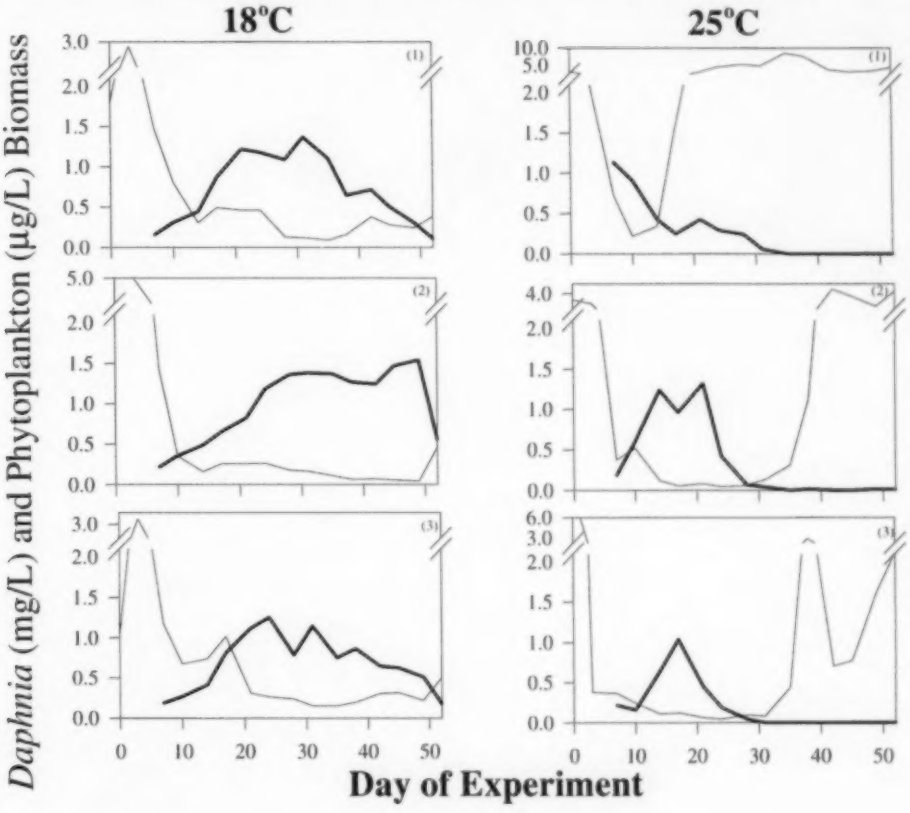
Fig. 5. Total *D. pulex* biomass (heavy lines) and phytoplankton biomass (as estimated by chl *a* concentration) (thin lines) for the three replicate tanks in the 18°C and 25°C three-level treatments (phytoplankton +*D. pulex* and competitive grazers + carnivore).



Watson and McCauley 1988; Watson et al. 1992). Finally, higher temperatures promote the growth of less edible species such as blue-greens (Patrick 1974). In light of these factors, we should expect the result that the recovery in phytoplankton represented an increase in less edible species that could not support the growth of *D. pulex* as observed in this study. The enumerated Lugol's samples showed that in the higher temperature treatment, the increase in algae accompanying the decline in *D. pulex* was more likely to consist of a large proportion of less edible species such as *Anabaena lemmermannii* var. *minor* and *Schizothrix calcicola*.

We have subsequently performed preliminary experiments identical to those discussed here for treatments with two and three trophic levels. These experiments were conducted in much smaller aquaria (100 L) and all inedible algae were completely excluded. Preliminary results show that (*i*) *M. ehrenbergii* again causes rapid *D. pulex* extinction but only at 25°C (confirming previous results) and (*ii*) in the absence of *M. ehrenbergii* and inedible algae at 25°C, replicate *D. pulex* populations persist for more than 100 days. This latter result demonstrates that *D. pulex* populations can persist at 25°C when the prey population consists entirely of edible phytoplankton and suggests that the buildup of inedible algae in the mesocosm experiments is a likely cause of assured *D. pulex* extinction.

To summarize, two hypotheses are proposed for the greater population instability at the higher temperature. Instability and extinction may have been caused by (*i*) the direct effect of temperature on vital rate parameters that destabilized the plant–herbivore interaction or (*ii*) an unfeasible plant–herbivore equilibrium with an increase in temperature owing to a shift in the algal community structure to less edible species. It remains to be determined which of these factors plays the greatest role in causing instability, although the evidence points to a combination of explanations. The greater instability could have arisen from a temperature-induced alteration of population vital rates to the point where extinction occurred by the starvation of *D. pulex*. Extinction was assured by the large-amplitude fluctuation in *D. pulex* and by a shift in algal community structure to less edible species that could not support *D. pulex* growth. This response to temperature was little influenced by community structure (i.e., the presence of herbivore competitors) or by the lengthening of the food chain. The presence of a dynamic invertebrate carnivore population only acted to increase the rapidity of extinction with an increase in temperature. Regardless of the exact mechanism, an important conclusion to draw is that increasing temperature leads to instability and decreased persistence in *D. pulex* – algal interactions, apparently independent of trophic structure.

Our results suggest implications of the temperature

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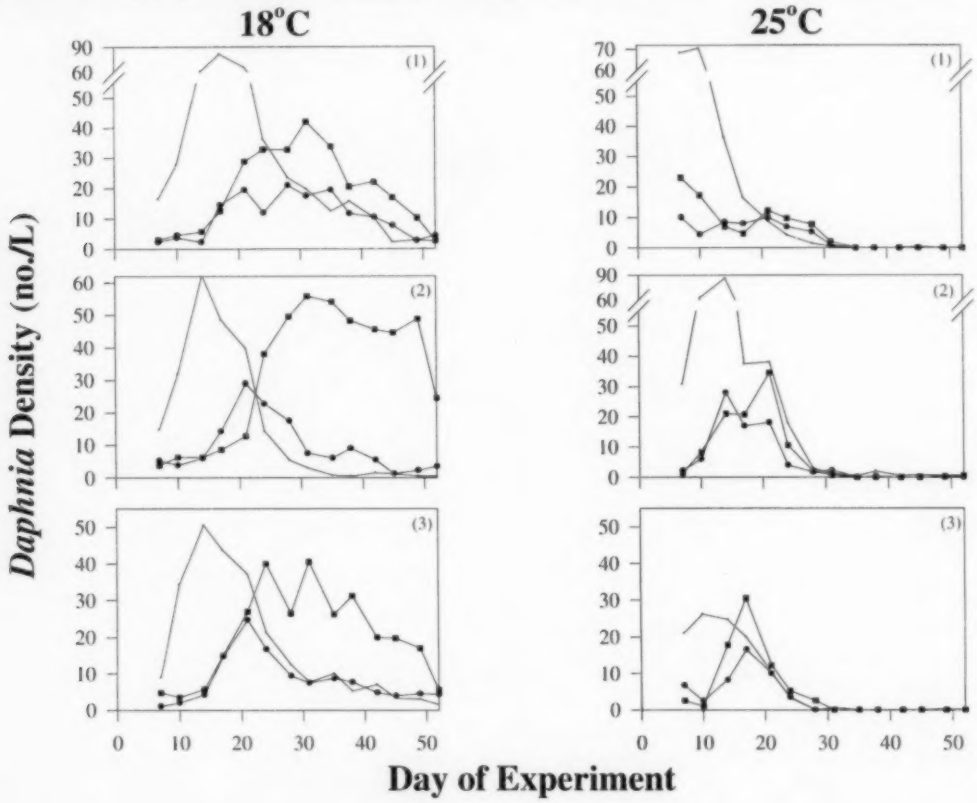
Fig. 6. *Daphnia pulex* density in the juvenile (thin lines), adolescent (●) and adult (■) size-classes for the three replicate tanks in the 18°C and 25°C three-level treatments (phytoplankton + *D. pulex* and competitive grazers + carnivore).



increases expected under current global warming scenarios for simple north temperate pond systems. Although we recognize that mesocosm systems are simplified versions of more complex systems, our results do account for some aspects of food web complexity, and these do not affect the major qualitative result. Our mesocosm results indicate that pond systems consisting of an array of herbivore species feeding on phytoplankton may lose their larger zooplankton herbivores as temperature increases, because of temperature-induced instability in the population dynamics, leading to extinction. The resultant communities observed in this study consisted of largely nuisance blue-green algae and small zooplankton herbivores. The complexity of the plankton communities (i.e., grazing competitor diversity) did not alter this important qualitative result. For systems with three trophic levels with an invertebrate carnivore that is dynamically coupled to the herbivore, the results are even more striking, with the shift to inedible algae and small zooplankton species occurring even more rapidly.

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Thyroid function and olfactory responses to L-alanine during induced smoltification in Atlantic salmon, *Salmo salar*

P.-P. Morin, T.J. Hara, and J.G. Eales

Abstract: For 5 weeks starting in mid-February we examined developmental correlations between external smolt features, plasma thyroid hormone levels, and olfactory responses in Atlantic salmon transferred from 0.9 to 11°C and exposed to a 16 h light : 8 h dark (16L) or an 8 h light : 16 h dark (8L) photoperiod. In 16L fish, external smolt features developed to 80% of full state, plasma L-thyroxine (T_4) surged at week 3, but there were no changes in plasma 3,5,3′-triiodo-L-thyronine, olfactory bulb electroencephalographic (EEG), or olfactory epithelium electro-olfactographic (EOG) activities in response to nasal stimulation with L-alanine (10^{-9} to 10^{-5} M). In 8L fish, external smolt features were arrested at 40% of full state, plasma T_4 showed no surge, EOG activity increased modestly, but EEG activity increased markedly at weeks 3 and 4. Thus, under the particular photoperiod and temperature conditions imposed in this study in February and March, enhanced olfactory activity can develop in premigratory Atlantic salmon independently of external smolt features or a significant surge in plasma T_4.

Résumé : Pendant 5 semaines, à partir de la mi-février, nous avons examiné les corrélations développementales entre les caractéristiques externes de smolt, les concentrations d'hormones thyroïdiennes dans le plasma et les réactions olfactives chez des saumons atlantiques transférés de 0,9 à 11°C et exposés à une photopériode de 16 h d'éclairement et 8 h d'obscurité (16L) ou de 8 h d'éclairement et 16 h d'obscurité (8L). Chez les poissons 16L, les caractéristiques externes de smolt ont atteint 80% du développement complet, la L-thyroxine (T_4) du plasma a marqué une poussée à la troisième semaine, mais on n'a pas observé de changement dans l'activité de la 3,5,3′-triiodo-L-thyronine du plasma, l'activité électro-encéphalographique (EEG) du bulbe olfactif ni dans l'activité électro-olfactographique (EOG) de l'épithélium olfactif en réaction à une stimulation nasale à la L-alanine (10^{-9}–10^{-5} M). Chez les poissons 8L, les caractéristiques externes de smolt se sont fixées à 40% du développement total, la T_4 plasmique n'a montré aucune poussée, l'activité EOG s'est accrue modestement, mais l'activité EEG a augmenté de façon marquée à la troisième et à la quatrième semaine. Dans les conditions particulières de photopériode et de température imposées au cours de cette étude réalisée en février et mars, il apparaît donc qu'une activité olfactive accrue peut se développer chez les saumons atlantiques en période pré-migratoire indépendamment des caractéristiques externes de smolt ou d'une poussée marquée de la T_4 plasmique.
[Traduit par la Rédaction]

Introduction

Prior to downstream seaward migration, juveniles of several anadromous salmonid fish undergo parr–smolt transformation (PST), which under natural conditions of temperature and photoperiod increments takes place progressively between January and May. This phase of development occurs when salmon attain a threshold size after 1 to several years of residence in fresh water. It involves temporally coordinated changes in morphology, physiology, and behavior (Hoar 1988). During PST, young salmon develop the ability to detect and remember the odor of the stream. This ability contributes to home-stream recognition during later upstream migration as potentially spawning adults (reviewed by Hasler and Scholz 1983). In laboratory-held Atlantic salmon (*Salmo salar*) induced to undergo PST by photoperiod and temperature manipulation, heart-rate conditioning to L-cysteine showed that odor learning associated with long-term memory occurs during a period of about 1 week (Morin et al. 1989*a*, 1989*b*). This coincides closely with the period when olfactory responses to the test substances, L-alanine and taurocholate, are maximal (Morin and Døving 1992; Morin et al. 1994).

During PST there are also changes in thyroid function, first described as enhanced histological activity of the subpharyngeal thyroid tissue of Atlantic salmon (Hoar 1939). This has been supported by Dickhoff et al. (1978), who found a pronounced surge in plasma L-thyroxine (T_4) concentration during PST. Elevation of plasma T_4 concentration has now been described for several salmonids during PST, including Atlantic salmon (Boeuf et al. 1989; Prunet et al. 1989; Morin et al. 1993). However, these changes in plasma T_4 concentration are not necessarily accompanied by comparable changes in plasma 3,5,3′-triiodo-L-thyronine (T_3) concentration, the presumed biologically active derivative of T_4. This is due possibly to alteration in thyroid hormone peripheral metabolism (Specker et al. 1984, 1992; Morin et al. 1993; Eales et al. 1993).

Several workers have suggested that changes in thyroid

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function may influence olfactory function and olfactory learning during PST (reviewed by Hasler and Scholz 1983). For example, olfactory recognition of a test substance was enhanced in juvenile coho salmon (*Oncorhynchus kisutch*) by increased thyroidal status owing to injection of thyroid stimulating hormone (Hasler and Scholz 1983). In support of this view, the period of enhanced odor learning and enhanced responsiveness to test odors in Atlantic salmon was determined using electrophysiological recordings from the olfactory bulb, and this period coincided with a transitory surge in histologically evaluated thyroidal status (Morin et al. 1989*b*, 1994). On the other hand, in these same studies, correlations between olfactory function and plasma T_4 or T_3 concentration were less evident. In a recent study we showed that treating advanced parr with T_4 did not increase olfactory function and in fact caused it to be depressed (Morin et al. 1995). In summary, there is some evidence, primarily correlative, suggesting relationships between thyroid function and olfactory function during PST. However, no clear-cut role of the thyroid hormones in influencing olfactory function in premigratory salmon has been established.

In this study we investigated the extent of developmental correlation between external smolt features, thyroid function, and olfactory activity. In mid-February, we exposed two groups of juvenile premigratory Atlantic salmon, previously acclimated at 0.9°C, to either a 16 h light : 8 h dark or an 8 h light : 16 h dark photoperiod at 11°C, which should advance or retard, respectively, the development of smolt features. During 5 weeks we then monitored development of external smolt characteristics, olfactory responses to L-alanine (10^{-9} to 10^{-5} M), and plasma T_4 and T_3 concentrations as indices of thyroid function. A close temporal correlation between changes in thyroid function and olfactory function, despite different rates of development of other aspects of PST, would support a thyroid role in influencing olfactory function. Electrophysiological responses to L-alanine were measured simultaneously at the olfactory epithelium (electro-olfactogram; EOG) and in the olfactory bulb (electroencephalogram; EEG), to determine changes in olfactory function at the levels of the primary receptor cells and the secondary olfactory bulbar neurons, respectively. Changes in function of the primary receptor cells have not been measured during PST. We chose L-alanine as test substance because it is highly stimulatory to the olfactory system of salmonids (Hara 1973) and because we wished to compare results with those of Morin and Døving (1992).

Methods

Fish maintenance

Atlantic salmon (age 20 months; body mass approximately 55 g) spawned from wild parents (Mitis River, Quebec) were raised at the Quebec Provincial Hatchery, Anse Pleureuse, Quebec, Canada, at 0.9°C (monthly average in February) under natural photoperiod before being flown to Winnipeg, Manitoba, on 13 February 1991. The fish were held in tanks receiving filtered and recirculated Winnipeg city water at 11 ± 0.5°C and were exposed to an 8 h light : 16 h dark (light period 08:00–16:00) photocycle. Every Saturday the water in the system was gradually replaced with fresh dechlorinated water. Each tank was illuminated by two 15-W incandescent bulbs set 76 cm above the water surface. During this initial acclimation period the fish were not fed. On 18 February the photocycle for one group of fish was adjusted to 16 h light : 8 h dark (light period 08:00–24:00) (16L group) while another group of fish was maintained under the 8 h light : 16 h dark regimen (8L group). The fish were fed to satiation twice each day (11:00 and 16:00) with 3-mm trout grower pellets (Martin Feed Mills, Elmira, Ont.). Fish were held under these conditions for 5 weeks and they were sampled routinely during weeks 2, 3, 4, and 5 as described below.

EEG and EOG measurements

On 5 consecutive days during each week, one fish was withdrawn per day at random from each of the two photoperiod conditions, and EEG and EOG activities were measured simultaneously according to the procedures of Morin et al. (1995). In brief, the olfactory epithelium was perfused by a constant water flow (12 mL/min) that could be interrupted and immediately replaced by a 10-s (2 mL) pulse of test odorant. The EOG responses to that odorant were recorded differentially from a glass capillary pipette (ca. 40-µm tip diameter) filled with saline–gelatin and bridged to an Ag–AgCl electrode, and electrical signals were amplified with a direct-current preamplifier, digitized, and analyzed on-line on a PC microcomputer. EEG activity was recorded through bipolar stainless steel electrodes (100-µm tip diameter) and amplified and integrated by an alternating-current preamplifier and integrator before being analyzed on-line on the PC microcomputer. The first fish was tested commencing at 09:30 and the second fish, at 13:30. All measurements were conducted at 11 ± 0.5°C.

Blood sampling

Following electrophysiological analysis, each fish was weighed and measured (fork length). At this time, a second but previously undisturbed fish from the same group was anesthetized with 2-phenoxyethanol (0.25 mL/L) and bled from caudal vessels using a heparinized syringe. The plasma was separated by centrifugation and stored at –70°C for later T_3 and T_4 radioimmunoassay (Omeljaniuk et al. 1984).

Smolt characteristics

Six externally visible smolt characteristics (loss of vertical parr marks, loss of back–belly contrast, loss of red spots on the flanks, development of black margins on the caudal fin, development of black margins on the pectoral fins, and development of black spots on the operculum) were assigned a numerical rank. The rankings for each character were summed with equal weightings to create an index of smoltification, varying from 0% (full parr state) to 100% (full smolt state) (Morin et al. 1993). The condition factor was calculated as $100 \times \text{mass (g)} / \text{length}^3 \text{ (cm)}$.

Data analysis

To fit the normal distribution curve, values of some variables were log or square-root transformed. Two-way analysis of variance (ANOVA) (groups × weeks) was used to test main differences or interaction effects that may exist in mass, body length, condition factor, and plasma T_4 and T_3 levels between 16L and 8L groups and among weeks. We also used one-way ANOVA to test specific differences that may exist among weeks for any particular group of fish. For this purpose, we were particularly interested in a posteriori comparisons between pairs of successive weeks using the Duncan's multiple range test. Two-way ANOVA (concentrations × weeks) was also used to examine specific differences in EEG and EOG activities among weeks and for a particular group of fish (8L or 16L group), when their olfactory epithelium was stimulated with different concentrations of L-alanine. Overall EEG and EOG responses to L-alanine (10^{-9} to 10^{-5} M) were also analyzed using a three-way multiple analysis of variance (MANOVA) (groups × weeks × concentrations of odorant) with repeated measures on concentrations. Procedure MANOVA for SPSS-PC was used to reveal main effects of experimental treatments on EEG and EOG activities. This statistical package offers the opportunity to choose a univariate or multivariate solution to treat

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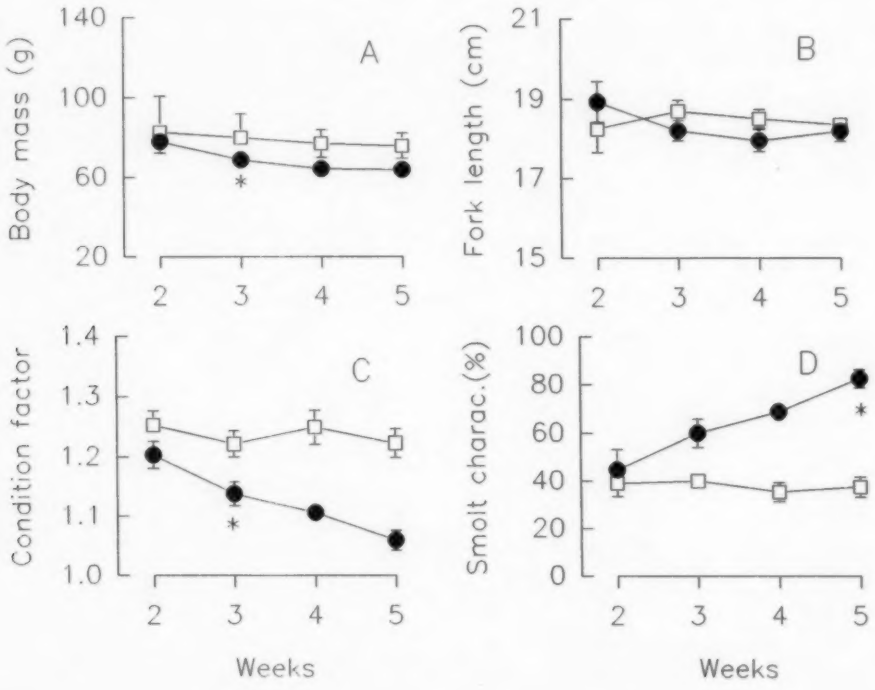
Fig. 1. Morphological changes (mean ± SE, $n = 7–10$) in Atlantic salmon held at 11°C under either a 16L (●) or an 8L photoperiod (□). (A) Body mass, showing differences between photoperiods ($p < 0.01$) and among weeks ($p < 0.05$). (B) Fork length. (C) Condition factor, showing the effect of photoperiod × weeks ($p < 0.05$) and differences between photoperiods within weeks 3, 4, and 5 ($p < 0.01$). (D) Smolt characteristics, showing the effects of photoperiod × weeks ($p < 0.01$) and between photoperiods within week 3 ($p < 0.05$) and weeks 4 and 5 ($p < 0.01$). An asterisk indicates a significant difference ($p < 0.05$) from the preceding week for the 8-h photoperiod (one-way ANOVA and Duncan's test).



factorial design with repeated measures. Our choice for using a univariate approach was based on Mauchly's test of sphericity, testing the hypothesis that the variances of all of the transformed variables for an effect be equal and that their covariances be zero (Morrison 1967).

Results

In 16L fish there was a small but significant decrease in body mass, a significant decrease in condition factor, and an increase in index of smolt characteristics, which at 5 weeks attained over 80% of the full smolt state (Fig. 1). In 8L fish there were no significant changes in body mass, body length, condition factor, or index of smolt characteristics, which was arrested at 40% of the full smolt state. There were no mortalities, and both 16L and 8L fish appeared to be in good health.

Plasma T_4 concentration was significantly higher overall in the 16L fish than in the 8L fish. When each group of fish was examined separately, plasma T_4 concentration increased significantly from week 2 to week 3 and then decreased gradually at weeks 4 and 5 in the 16L fish; despite a suggestion of an increase in plasma T_4 concentration at week 3, there was no significant temporal change in the 8L fish (Fig. 2A). Plasma T_3 concentration did not change significantly over the course of the experiment for either the 16L or 8L fish and was not influenced by photoperiod (Fig. 2B).

Representative integrated EEG responses of the olfactory bulb to L-alanine at 10^{-9} and 10^{-5}M concentrations are shown for 8L fish tested at weeks 3 and 4 (Fig. 3). With the exception of 8L fish (week 2), the EEG response to L-alanine was generally concentration dependent over the range 10^{-9} to 10^{-5}M (Fig. 4). There was a peak in the EEG response at week 4 for the 8L fish but no significant change for the 16L fish. For the 8L fish, the EEG response to 10^{-9} M L-alanine during week 4 equalled the response to 10^{-5} M L-alanine at week 2, emphasizing the major increase in olfactory function at week 4. Only at week 4 did the EEG response of 8L fish to 10^{-9} M L-alanine differ significantly from that of controls. However, some changes in olfactory function may occur as early as week 2. At this time the EEG response to logarithmic increments of L-alanine was not linear. It reached a plateau at 10^{-8} M L-alanine.

Representative EOG responses of the olfactory epithelium at 10^{-9} and 10^{-5} M L-alanine concentrations are shown for 8L fish (Fig. 5). The EOG response to L-alanine was concentration dependent over the range 10^{-9} to 10^{-5} M (Fig. 6). There was no change in the overall EOG response from week 2 to week 5 and no difference between 8L and 16L fish. However, for the 8L fish during weeks 4 and 5, the EOG response to 10^{-9} M L-alanine was significantly greater than the control.

Discussion

Our objective was to determine the extent of developmental

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Fig. 2. (A) Plasma T_4 levels (mean ± SE, $n = 4–5$) in Atlantic salmon held at 11°C under either a 16L (●) or an 8L photoperiod (□). Differences exist between photoperiods ($p < 0.01$) and among weeks ($p < 0.01$). A difference exists among weeks ($p < 0.05$) for 16L fish. The asterisk indicates a difference between weeks 2 and 3 ($p < 0.01$) for the 16L fish. (B) Plasma T_3 levels (mean ± SE, $n = 4–5$) in Atlantic salmon held at 11°C under either a 16L (●) or an 8L photoperiod (□). No significant differences existed between photoperiods or among weeks.

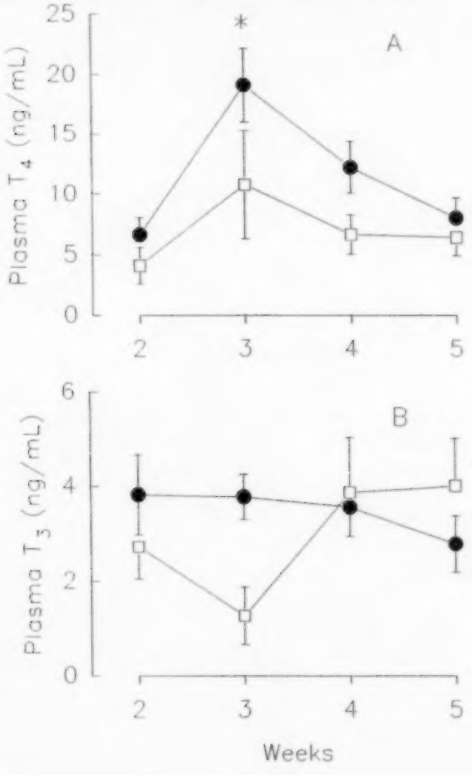
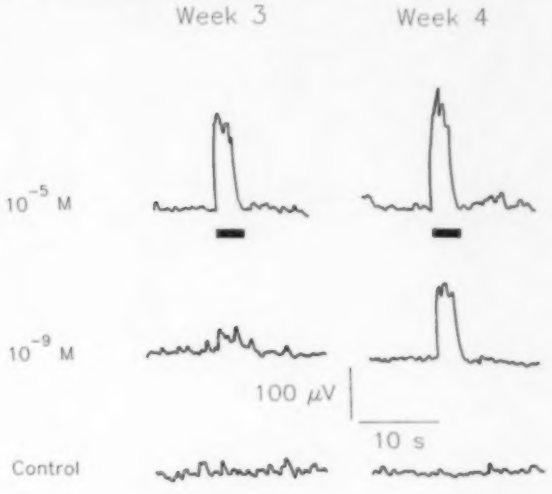


Fig. 3. Representative integrated EEG recordings following stimulation of the olfactory epithelium with 10^{-9} and 10^{-5} M L-alanine in Atlantic salmon held at 11°C under an 8L photoperiod, and examined at week 3 or week 4.



correlation between changes in external smolt characteristics, thyroid function (plasma T_4 surge), and olfactory function (EEG and EOG responses to L-alanine) in premigratory salmon under photoperiod conditions (16L or 8L) designed to alter the rate of PST development. We have shown that the smolt characterisics, plasma T_4 surge, and aspects of olfactory function are all influenced by photoperiod but that development of enhanced olfactory activity is not closely linked to other smolt features and need not be preceded by an increase in plasma T_4 concentration.

The 16L photoperiod induced a decline in condition factor and promoted the development of other externally visible features of PST, so that by week 5 the smolt index exceeded 80% for 16L fish but remained below 40% for 8L fish. These observations are consistent with previous studies on the influence of extended photoperiod (Morin et al. 1989*a*; Stefansson et al. 1991; Morin and Døving 1992) or simulated natural photoperiod (Robertson and Bradley 1991; Rourke et al. 1991) on the condition factor and development of external smolt characteristics in Atlantic salmon. Body mass did not increase during the experiment and in 16L fish it fell slightly. Comparable small mass losses have been observed in premigratory Atlantic salmon held in captivity in heated-water systems (Dickhoff et al. 1989).

Under the 8L photoperiod there was no significant change in plasma T_4 concentration, but at week 3 under the 16L photoperiod there was a significant T_4 surge, typical in its extent of that for Atlantic salmon undergoing PST under natural conditions (Dickhoff et al. 1989; Boeuf et al. 1989; Prunet et al. 1989) or under conditions of extended day length with constant temperature (Morin et al. 1989*a*, 1989*b*, 1993, 1994; Morin and Døving 1992). In agreement with some previous PST studies (Folmar and Dickhoff 1981; Morin et al. 1989*b*, 1993), the increase in plasma T_4 concentration was not accompanied by an increase in plasma T_3 concentration. This may reflect T_4-induced alterations in deiodinase functions that regulate conversion of T_4 to T_3 and T_3 degradation (Morin et al. 1993). In Atlantic salmon parr, for example, an experimentally induced physiological surge in plasma T_4 concentration decreased hepatic and brain formation of T_3, enhanced hepatic degradation of T_3, and depressed the plasma T_3 concentration (Morin et al. 1995). However, plasma T_3 levels may not necessarily reflect tissue T_3 levels. During PST, the brain T_3 concentration of smolting coho salmon decreased markedly and disproportionately to the plasma T_3 concentration (Specker et al. 1992).

There were no significant developmental changes in the olfactory bulb EEG response to L-alanine for the 16L fish, but for the 8L fish, EEG activity increased from week 2 to week 4. This period of heightened EEG activity in 8L fish may correspond to the window of enhanced olfactory activity documented previously during week 4 in premigratory Atlantic salmon (Morin and Døving 1992; Morin et al. 1994). However, the EEG responses to L-alanine, expressed as a ratio to the control level, exceeded those of Morin and Døving (1992). This may be due to the recording electrodes being placed in a more posterior position (adjacent to the telencephalon) in the

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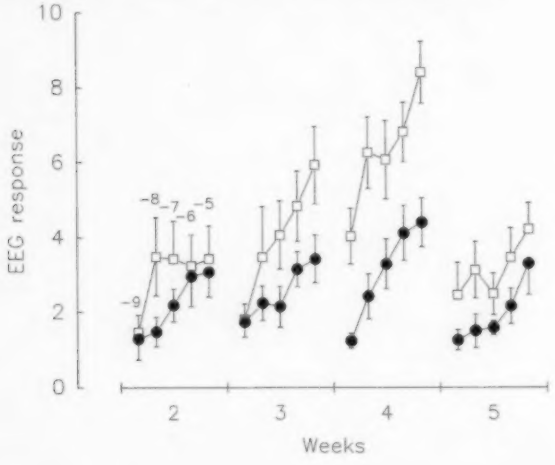
Fig. 4. Mean (±SE, $n = 3$–5) EEG response (expressed as the ratio to the control value) for five concentrations of L-alanine (10^{-9} to 10^{-5} M) in Atlantic salmon held at 11°C under a 16L (●) or 8L (□) photoperiod. Three-way MANOVA (univariate solution) indicated differences between photoperiods ($p < 0.01$), among weeks ($p < 0.01$), and among concentrations ($p < 0.01$). The overall EEG response was greater at week 4 than at week 3 ($p < 0.05$) and lower at week 5 than at week 4 ($p < 0.01$) under the 8L photoperiod.



present study. In rainbow trout (*Oncorhynchus mykiss*), Hara et al. (1973) compared EEG recordings following nasal stimulation with L-serine and found greater responses from the posterior than from the anterior region of the olfactory bulb.

Likewise, there were no significant developmental changes in the EOG response for the 16L fish, but for the 8L fish, increases in the EOG response to 10^{-9} M L-alanine occurred at weeks 4 and 5. At week 4 in particular there may be greater olfactory epithelial sensitivity to L-alanine. However, the developmental changes in the EOG response were less marked than those in the EEG response. This difference may be due in part to neural convergence between the primary and secondary olfactory neurons.

We showed recently (Morin et al. 1995) that treatment of premigratory Atlantic salmon with T_4 did not enhance EEG or EOG responses to L-alanine and in fact decreased the EEG response. Thus, as shown for the 8L fish in the present study, an elevation in plasma T_4 level may not be required to elevate olfactory activity, and instead, as shown for the 16L fish in the present study, it may act to suppress olfactory activity. There are changes in several other hormones (e.g., growth hormone and steroid hormones) during PST (Hoar 1988), and these may be involved in the development of enhanced olfactory activity. Instead, the surge in plasma T_4 concentration in the advanced stages of PST may serve to decrease brain olfactory function and terminate the period of olfactory learning prior to downstream movement to different chemical environments (Morin et al. 1995).

In contrast to the present study, previous studies on premigratory juvenile Atlantic salmon consistently demonstrated development of a brief window of enhanced olfactory activity under conditions of extended photoperiod and elevated water temperature, and furthermore, the increased olfactory activity

Fig. 5. Representative EOG recordings following stimulation of the olfactory epithelium with 10^{-9} and 10^{-5} M L-alanine (C, control) in Atlantic salmon held at 11°C under an 8L photoperiod and examined at week 3 or week 4.

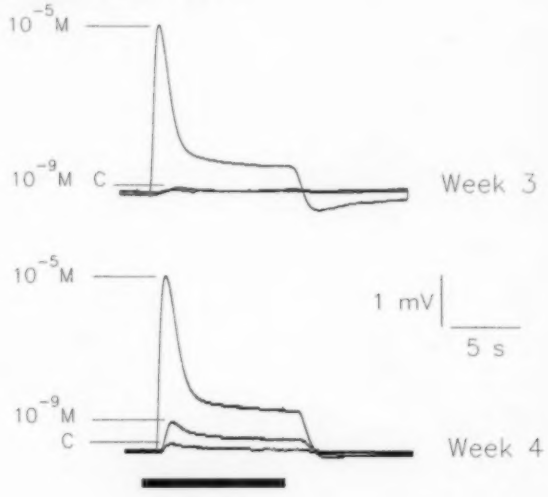
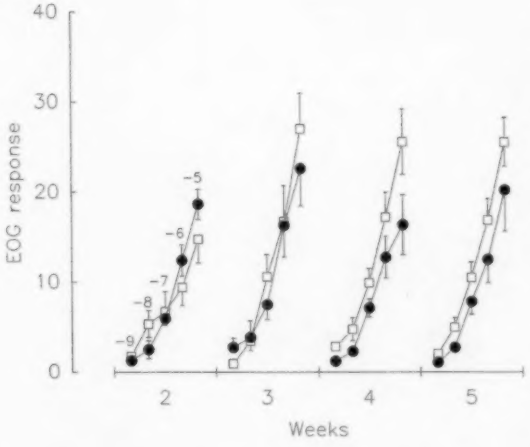


Fig. 6. Mean (±SE, $n = 3$–5) EOG response (expressed as the ratio to the control value) for five concentrations of L-alanine (10^{-9} to 10^{-5} M) in Atlantic salmon held at 11 C under a 16L (●) or 8L (□) photoperiod. Three-way MANOVA (multivariate solution) indicated differences between concentrations ($p < 0.01$), but no significant difference among weeks or between photoperiods ($p > 0.05$).



was correlated with enhanced thyroid function (Morin et al. 1989*a*, 1989*b*, 1994; Morin and Døving 1992). However, in previous studies, environmental changes in temperature and photoperiod were imposed at the end of December when fish were in the very early stages of PST, corresponding to a 10% development of external smolt characteristics; in the present study, comparable environmental changes were imposed on 18 February, corresponding to a 40% development of external smolt characteristics. Thus, there is a major difference in the timing of the environmental transition relative to PST

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development and to the normal period of migration in April. In the 16L fish used in the present study, the acceleration of events relatively close to the migration period may have so compressed the window for neural development that changes in function of the olfactory bulb were never expressed or were not detected. However, in the 8L fish where the rate of development was less rapid, the window of enhanced olfactory activity could still be expressed and detected.

Olfactory activity was determined from the EEG and EOG responses to L-alanine over the concentration range 10^{-5} to 10^{-9} M. We have assumed that the response to L-alanine over this concentration range is a reliable index of overall olfactory activity. We cannot exclude the possibility that responses might have differed to natural waterborne chemicals detectable at lower concentrations. However, responses of Atlantic salmon to L-alanine and taurocholate are similar (Morin and Døving 1992; Morin et al. 1994).

The salmon used in this study were flown from Quebec and held in our local water source for 2 weeks prior to any EEG or EOG measurements. Thus, changes in water quality, temperature, or transition from hatchery tanks to laboratory tanks may have influenced either the olfactory responses or thyroid function. Plasma T_4 concentrations increase in Pacific salmon during PST following their transfer to a new water source (Grau et al. 1985; Lin et al. 1985; Hoffnagle and Fivizanni 1990).

In summary, the 16L photoperiod, while inducing external smolt features and the surge in plasma T_4 concentration in premigratory Atlantic salmon, did not influence significantly olfactory activity in response to L-alanine. On the other hand, under the 8L photoperiod, in which the development of external smolt features and the surge in plasma T_4 were suppressed, there was a marked transitory increase in olfactory bulbar response to L-alanine. Our results support the emerging view that the suite of developmental changes generally grouped under the umbrella of PST represent phenomena that are not necessarily synchronous and may not be linked in their control. For example, early manifestations of PST, such as increased Na^+,K^+-ATPase activity and decreased condition factor, may start to develop in January, well ahead of the phenomena considered here (Boeuf et al. 1989; Robertson and Bradley 1991; Stefansson et al. 1991). We conclude that the window of enhanced olfactory activity may not necessarily be linked to development of external smolt characteristics, and a surge in plasma T_4 concentration may not necessarily be required to induce measurable enhanced olfactory activity in premigratory salmon.

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Effect of stream acidification and inorganic aluminum on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York

Barry P. Baldigo and Peter S. Murdoch

Abstract: Juvenile brook trout (*Salvelinus fontinalis*) were exposed in cages to fluctuating chemical conditions in four Catskill Mountain streams during the spring and fall of 1989 and the spring of 1990. Specific chemical constituents and characteristics of acidic episodes that correlated with increased fish mortality were identified. Mortality increased during acidic episodes in one poorly buffered stream when inorganic monomeric aluminum (Al_{im}) concentrations increased; mortality was low in three other streams during acidic episodes of shorter duration and smaller magnitude than measured in the poorly buffered stream. Variation in mortality was attributed primarily to differences in concentrations of both Al_{im} and dissolved organic carbon. Linear and logistic regression analyses indicate that either mean or median Al_{im} concentrations could account for 73–99% of the variability in mortality. Regression analyses suggest that mortality was highly related (in order of importance) to Al_{im}, pH, dissolved organic carbon, calcium, and chloride concentration. Brook trout mortality was also highly related to durations of exposure above 0.225 and 0.250 mg/L Al_{im} during test periods. Characteristics of acidic–Al_{im} episodes that are critical to mortality of caged brook trout appear to be (*i*) Al_{im} concentrations of at least 0.225 ± 0.025 mg/L and (*ii*) exposure to these toxic Al_{im} concentrations for at least 2 days.

Résumé : Des juvéniles d'ombles de fontaine (*Salvelinus fontinalis*) ont été exposés dans des cages à des conditions chimiques fluctuantes dans quatre cours d'eau des monts Catskill pendant le printemps et l'automne de 1989 et le printemps de 1990. Les constituants chimiques spécifiques et les caractéristiques des épisodes acides qui étaient corrélés à une hausse de la mortalité des poissons ont été identifiés. La mortalité a augmenté pendant des épisodes acides dans un cours d'eau à faible pouvoir tampon quand les concentrations d'aluminium inorganique monomère (Al_{im}) augmentaient; la mortalité était faible dans trois autres cours d'eau pendant des épisodes acides de plus courte durée et de moindre ampleur que dans le cours d'eau à faible pouvoir tampon. La variation de la mortalité a été attribuée avant tout à des différences dans les concentrations d'une part d'Al_{im}, et d'autre part de carbone organique dissous. Des analyses de régression linéaire et logistique indiquent que les concentrations moyennes ou médianes d'Al_{im} pouvaient expliquer 73–99% de la variabilité de la mortalité. Les analyses de régression permettent de penser que la mortalité était fortement reliée (par ordre d'importance) à Al_{im}, au pH, au carbone organique dissous, au calcium et au chlore. La mortalité de l'omble de fontaine était aussi fortement liée à la durée de l'exposition au-dessus de 0,225 et 0,250 mg/L Al_{im} pendant les périodes de test. Les caractéristiques des épisodes d'acidité–Al_{im} qui sont critiques pour la mortalité des ombles de fontaine en cage semblent être (*i*) des concentrations d'Al_{im} d'au moins 0,225 ± 0,025 mg/L, et (*ii*) l'exposition à ces concentrations toxiques d'Al_{im} pendant au moins 2 jours. [Traduit par la Rédaction]

Introduction

The effects of acidic precipitation on aquatic ecosystems in much of the northeastern United States have been well documented (see reviews by Haines and Baker 1986; Baker et al. 1990); however, only a few studies have described stream acidification in the Catskill Mountains (Stoddard and Murdoch 1991; Shultz et al. 1993; Murdoch and Stoddard 1993; Wigington et al. 1996) or documented the effects of chronic and episodic acidification on survival of native brook trout (*Salvelinus fontinalis*) in streams of the region (Murdoch et al. 1991; Van Sickle et al. 1996). In addition, the specific chemical constituents and the magnitude, duration, and frequency characteristics of stochastic concentrations that cause fish mortality in natural streams are also not completely understood (Baker et al. 1990).

Relations between pH and survival of fish in the laboratory and in selected natural systems have been studied over the past three decades (see review by Baker et al. 1990). Relations between mortality of native fish (in situ) and specific chemical constituents (other than pH) and their temporal characteristics in naturally acidified streams elude precise definition, however, because (*i*) water chemistry and toxicity vary with stream discharge and season, and (*ii*) complex interactions among biotic and abiotic factors can alternately increase and (or) decrease the detrimental effects of toxic waters on individual fish (Baker et al. 1990).

Watersheds and streams within the Catskill Mountains of southeastern New York (Fig. 1) receive atmospheric precipitation that is highly acidic (Barchet 1991; Stoddard and Murdoch 1991). Elevated atmospheric deposition of sulfuric and nitric acids in Catskill watersheds appears to be the basis for both chronic and episodic acidification in some headwater

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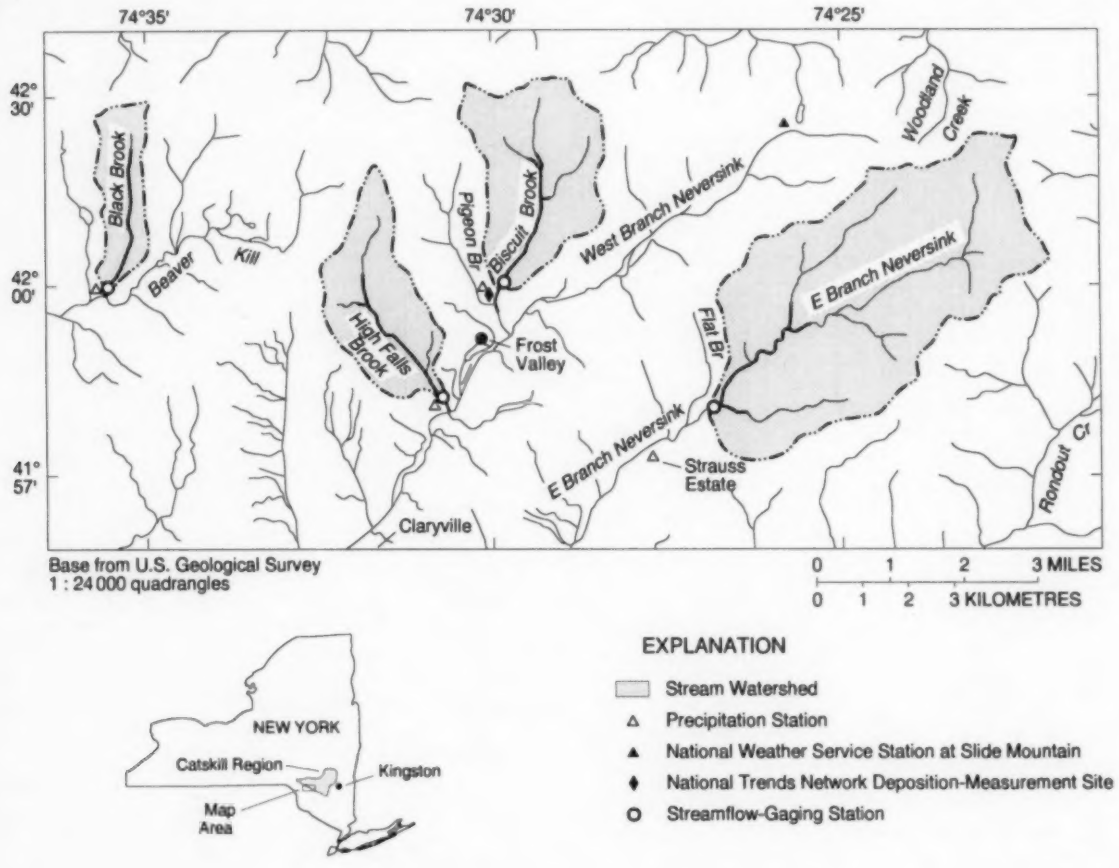
Fig. 1. Location of study area and of the four study streams in the Catskill Mountains of New York State. (Modified from Murdoch et al. 1991, Fig. 2.)



streams (Murdoch and Stoddard 1993). During 1989, the Catskill region received 21.9 kg/ha of wet NO_3^- deposition and 25.7 kg/ha of wet SO_4^{2-} deposition (Wigington et al. 1996). Although well-buffered soils prevent acidification of most streams throughout the Catskill region, parts of the Neversink River (Fig. 1) and other small headwater systems drain poorly buffered soils and have become acidified (Rich 1934; Stoddard and Murdoch 1991; Shultz et al. 1993). The wide range of acidic conditions that prevail in the Neversink River and adjacent Delaware River basins provide an ideal setting to study how chemical constituents and temporal characteristics affect fish mortality.

In 1988, the U.S. Environmental Protection Agency began the Episodic Response Project (ERP) (Wigington et al. 1993) to document the effects of episodic acidification on aquatic systems. The ERP was designed to describe and quantify the hydrology, chemistry, and biology of 13 headwater streams within mountainous regions of west-central Pennsylvania and in the Adirondack and Catskill mountains of New York state. One objective of the project was to identify which constituents and acidic-episode characteristics (frequency, magnitude, duration) were most highly related to brook trout mortality and to the decline of their natural populations in affected streams. The U.S. Geological Survey (USGS) coordinated ERP field research in the Catskill Mountains, and general results were summarized by Wigington et al. (1993). This paper presents results from the Catskill research and evaluates relations among selected chemical constituents, characteristics of their fluctuating concentrations, and mortality of caged native brook trout in four headwater streams during 1989–1990.

Study sites

The four streams chosen for the ERP investigation in the Catskill Park are about 50 km west of Kingston, N.Y. (Fig. 1). Biscuit Brook and High Falls Brook are tributaries to the West Branch Neversink River at Frost Valley; the East Branch Neversink site is about 5 km east of Frost Valley; and Black Brook is a tributary to the Beaver Kill in the Delaware River basin and is about 8 km west of Frost Valley. The drainage areas for Black Brook, High Falls Brook, Biscuit Brook, and East Branch watersheds are 3.7, 7.1, 9.9, and 23.1 km^2, respectively. The four streams were selected for study on the basis of (*i*) extensive data from long-term chemical monitoring at Biscuit Brook, (*ii*) proximity of a field laboratory (at Frost Valley), and (*iii*) the wide range in base-flow pH, acid neutralizing

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capacity (ANC), and total dissolved aluminum (Al_{td}) concentrations.

The climate of the Catskill Mountain region is classified as humid continental and is subject to extreme seasonal changes in temperature; the average annual temperature during 1951–1975 at a National Weather Service (NWS) station near the southeastern edge of the Catskill Park was about 9°C (Tornes 1979). The average annual snowfall during the same period was about 173 cm (Tornes 1979). The average annual rainfall during 1950–1982 at the NWS Slide Mountain station, 8 km northeast of Frost Valley (Fig. 1) was estimated to be 157 cm (Murdoch et al. 1991). Precipitation during 1989 at the National Trends Network deposition-monitoring station, adjacent to the Biscuit Brook site, was about 142 cm; volume-weighted pH of precipitation during 1989 and the during the previous 7 years was approximately 4.3 (Barchet 1991; Murdoch and Stoddard 1993). Regional and local precipitation chemistry is described in greater detail by Murdoch et al. (1991) and Barchet (1991). The physiography, geology, and soil classification of the Catskill region are summarized by Murdoch and Stoddard (1993) and Wigington et al. (1996) and discussed in detail by Tornes (1979), Rich (1934), Way (1972), and Ethridge (1977).

Many low-order watersheds of the Neversink River basin have steep gradients, thin soils, and extensive bedrock outcrops that provide rapid runoff, short residence time for subsurface water, and negligible buffering; thus, they are moderately to extremely acidic (Murdoch et al. 1991). Black Brook, a headwater stream in the adjacent Delaware River basin, was the primary reference stream in this study; ANC was well above zero at all times. High Falls Brook acted as a second reference stream because it underwent no periods of negative ANC, although ANC often approached zero. Biscuit Brook was selected as an episodically acidified site because ANC was generally above zero during base flow but often below zero during high flows. The East Branch Neversink was a chronically acidified stream; ANC values seldom exceeded zero, even during base-flow periods.

Methods

Water quality and discharge on the four streams (Fig. 1) were monitored from November 1988 through June 1990. Stream water was sampled weekly at each station and more frequently during changes in flow and during toxicity tests. Juvenile brook trout, native to the West Branch of the Neversink River and Pigeon Brook, were exposed (in cages) to natural conditions at each site for periods as long as 36 days during the spring and fall of 1989 and the spring of 1990. Field operations and quality-assurance procedures summarized here are described in detail by Wigington et al. (1993). Stream stage was measured and recorded every 15 min at each site by a submersible pressure transducer and electronic data logger. Discharge was measured twice monthly and used to generate stage-to-discharge ratings and 15-min discharge data for each stream in accordance with standard USGS methods (Rantz 1983). At each site, data loggers were programmed to instruct automated samplers to collect water samples at specified increments of rising and falling stage during periods of changing discharge. Routine water samples were collected weekly or biweekly from each site and filtered and preserved within 24 h; automated samples were generally processed within 3 days of collection.

From 50 to 80 routine water samples were collected at each stream during 1989 and during the first 5 months of 1990. A variable number of additional water samples were collected automatically at each stream during periods of high discharge and during toxicity tests. Total monomeric aluminum (Al_{tm}) and organic monomeric aluminum (Al_{om}) measurements were made on only about 10% of the routine samples because analytical costs and processing times were prohibitive. The actual numbers of routine Al_{tm}, Al_{om}, and inorganic monomeric aluminum (Al_{im}) measurements per stream were from 0 to 18 because the established criterion for processing monomeric Al aliquots was a water pH less than 5.5, which was seldom met at Black Brook and High Falls Brook.

The mean, median, minimum, maximum, and standard deviation (SD) for most chemical constituents were calculated for each site from the routine water samples. Statistics for chemical constituents during toxicity tests were obtained from all water samples collected during respective periods of exposure at each site. Statistics for monomeric-aluminum species (Al_{tm}, Al_{om}, and Al_{im}) at Black Brook were estimated from the annual flow record and the relations between the concentration of each of the three species and discharge because (*i*) no monomeric Al analyses were conducted on routine samples from this site; (*ii*) measured monomeric Al concentrations were low, typically near detection limits at this site; and (*iii*) only 10 monomeric Al analyses were performed at Black Brook.

We present results of 20 toxicity tests that used only native brook trout from a common source (West Branch of the Neversink River) and a small tributary (Pigeon Brook) (Fig. 1). The mortality of caged fish was measured at each stream during three periods: April 4 through June 24, 1989; October 4 through November 30, 1989; and March 9 through May 6, 1990. Tests were terminated and restarted after mortality exceeded either 20% at Black Brook or 90% at the other three sites; therefore, the exposure durations ranged from 11 to 36 days. Young-of-the-year brook trout (age 0+) were used in all tests; during spring periods, tested individuals were almost age 1+.

In each set of tests, brook trout were collected, placed into a large holding chamber at High Falls Brook for 24–48 h, then selected randomly for tests at each of the four study streams. For each test and stream, 20 or 21 brook trout were separated into three to five plastic, screen-sided jars that were in turn placed in cages near streamflow-gauging sites in accordance with techniques described by Johnson et al. (1987). Fish survival was checked and recorded every day or two during testing. Fish lengths ranged from 41 to 91 mm and averaged 71.5 ± 7.5 mm (mean ± SD). Fish weighed from 1 to 6 g and averaged 2.8 ± 0.9 g. Total lengths and mass did not differ ($p \leq 0.0003$) among streams or test periods, but mean lengths were 4–6 mm greater during spring tests than during fall tests.

Simple linear and logistic regression analyses were used to define relations between brook trout mortality (percent mortality at the end of each test) and estimates of mean pH, Al_{td}, Al_{tm}, Al_{om}, Al_{im}, Ca^{2+}, NO_3^-, SO_4^{2-}, Cl^-, and dissolved organic carbon (DOC) concentrations from all water samples collected at each stream during toxicity-test periods. Constituent means (and their median, maximum, and minimum statistics) that were related to percent mortality ($p \leq 0.05$) and those potentially related to either Al_{im} concentration or to fish sensitivity were then analyzed through stepwise multiple regression techniques. In stepwise (forward and backward) multiple regression analyses, independent regressors were judged to be potentially important and thus useful predictors of brook trout mortality if, after inclusion in the equation, the standard deviation of the residuals (SDR; equivalent to the root mean square error), decreased by at least one unit and the F values remained significant ($p \leq 0.05$). The rank or importance of each constituent to brook trout mortality level was gauged by (*i*) the probability of a legitimate association; $p \leq 0.05$ (significant t and F values), (*ii*) the coefficient of determination (r^2) or the adjusted r^2 for multiple-linear and logistic regressions, and (*iii*) the SDR.

Analyses of relations between statistics for individual chemicals and mortality levels assume that single constituents affect brook trout mortality independent of other constituents, whereas multiple-regression analyses assume that more than one variable affects fish mortality. All

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Table 1. Physical characteristics and concentrations of selected constituents for the four streams in the Catskill Mountains, N.Y., 1989–1990.

(A) Geographic characteristics

	Black Brook	High Falls Brook	Biscuit Brook	East Branch Neversink
Gauge latitude (N)	42°00′42″	41°58′40″	41°59′43″	41°58′01″
Gauge longitude (W)	78°36′13″	71°31′21″	74°30′05″	74°26′54″
Gauge elevation (m)	681	590	490	651
Drainage area (km^2)	3.65	7.10	9.84	23.1
Quadrangle	Seager	Claryville	Claryville	Peekamoose

(B) Water quality characteristics

	Black Brook				High Falls Brook				Biscuit Brook				East Branch Neversink			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
pH	6.53	0.32	5.44	7.04	6.48	0.34	5.40	7.08	5.99	0.34	4.97	6.50	4.85	0.16	4.27	5.35
ANC	95.8	38.1	17.1	175.9	87.4	36.8	11.5	171.8	24.1	12.1	–6.3	53.1	–8.1	6.9	–45.3	17.5
Al_{im}	0.006	na	0	0.072	0.017	0.018	0	0.08	0.077	0.066	0	0.243	0.211	0.081	0	0.639
Al_{tm}	0.046	na	0.028	0.179	0.073	0.003	0.010	0.203	0.125	0.079	0.015	0.363	0.262	0.085	0.051	0.746
Al_{om}	0.026	na	0.031	0.114	0.056	0.016	0.020	0.182	0.062	0.020	0.017	0.141	0.060	0.032	0.016	0.310
Al_{td}	0.061	0.086	0.002	0.539	0.043	0.072	0.001	0.773	0.055	0.046	0.002	0.332	0.217	0.080	0.016	0.737
Ca^{2+}	4.51	0.57	2.73	5.90	4.13	0.60	1.87	5.55	2.77	0.27	1.96	3.96	1.38	0.16	0.75	2.39
DOC	1.99	1.59	0.66	8.07	2.06	1.14	0.28	7.96	2.01	0.92	0.34	7.51	2.31	1.14	0.29	11.67
Mg^{2+}	0.60	0.08	0.38	0.80	0.55	0.07	0.28	0.73	0.61	0.06	0.38	0.77	0.62	0.08	0.34	0.97
Na^{+}	0.29	0.12	0.13	0.89	0.32	0.06	0.18	1.42	0.34	0.09	0.19	0.87	0.34	0.06	0.16	2.00
K^{+}	0.27	0.10	0.18	1.05	0.25	0.07	0.13	0.90	0.24	0.06	0.18	0.83	0.27	0.08	0.17	0.65
SO_4^{2-}	6.36	0.49	4.80	7.04	6.14	0.53	4.38	7.00	6.12	0.43	4.69	7.00	5.54	0.27	4.12	6.28
NO_3^{-}	3.01	0.99	0.59	6.69	2.45	1.11	0.99	6.59	2.61	1.21	0.69	7.99	2.25	1.10	0.63	6.57
Cl^{-}	0.57	0.08	0.19	0.82	0.58	0.08	0.26	0.83	0.62	0.08	0.35	0.80	0.55	0.05	0.36	0.82
Si	2.29	0.34	0	3.19	2.26	0.44	0.96	3.71	2.15	0.39	1.00	2.94	2.28	0.41	0.87	2.85
Flow	0.13	0.14	0.01	1.68	0.28	0.28	0.03	2.38	0.34	0.51	0.02	10.02	1.41	2.57	0.10	28.27

Note: Most physical information is from Murdoch et al. (1991). Flow values (m^3/s) were derived from hourly 1989 flow data. Chemical data were calculated from weekly water samples collected from January 1989 to June 1990. Values of Al_{tm}, Al_{om}, and Al_{im} at Black Brook were estimated from calculated hourly data. Concentrations of all chemical constituents, except acid neutralizing capacity (ANC) in microequivalents per litre and pH, are in milligrams per litre. DOC, dissolved organic carbon; Al_{im}, inorganic monomeric aluminum; Al_{tm}, total monomeric aluminum; Al_{om}, organic monomeric aluminum; Al_{td}, total dissolved aluminum. na, not available.

analyses assume that the residuals are normally distributed and their variances are homeoscedastic. Although these assumptions are often strained, even when normalized or transformed data are used, the analyses rank the importance of specific constituents to fish mortality, and identify interactions among constituents that could indirectly affect both water toxicity and brook trout sensitivity. Logistic regression analyses assume that mortality data are binomially distributed and that the observed response of fish mortality to a linear toxicant gradient asymptotically approaches the 0 and 100% level for low and high toxicant concentrations, respectively.

Hourly Al_{im} concentrations were estimated for each site (during test periods) to assess the effect of episodic increases in Al_{im} concentration on fish mortality. These data were calculated from mean hourly discharge and the relations between stream discharge and either (*i*) measured Al_{im} concentrations or (*ii*) an intermediate factor, such as pH, that is highly correlated with both discharge and Al_{im}. The calculated hourly Al_{im} record for each stream and each exposure period was used to summarize magnitude, duration, and frequency statistics for Al_{im} concentrations that fluctuated (Al_{im} episodes) in relation to periods of base flow and episodic acidification. In this paper, the term Al_{im} episode refers to a rapid and often sustained increase in ambient concentration of Al_{im} in stream waters; it always occurs in conjunction with acidic episodes and discharge events. Regression analyses were then used to relate percent brook trout mortality to the magnitude and temporal-Al_{im} statistics.

Results

Stream hydrology and chemistry

The mean hourly discharge of each of the four streams during the study period ranged from 0.13 to 1.41 m^3/s (Table 1) and is positively related to watershed area. Large fluctuations in discharge occurred throughout toxicity-test periods at each site (Fig. 2).

The degree of acidification (stream pH) generally reflected ANC values across study sites. Mean ANC in Black Brook and High Falls Brook was about 90 μequiv./L and never dropped below 10 μequiv./L. Mean ANC in Biscuit Brook was 24.1 μequiv./L; negative ANC values occurred only during very high flows. Mean ANC for the East Branch Neversink River was –8.1 μequiv./L; severe pH and ANC depressions were recorded frequently in this stream throughout the study. Mean pH ranged from a low of 4.85 in the chronically acidified stream (East Branch Neversink) to a high of 6.53 at the primary reference stream (Black Brook). Water pH was seldom below 6.0 at Black Brook and at the second reference stream (High Falls Brook), rarely below 5.5 at the episodically acidified stream (Biscuit Brook), and rarely exceeded 5.0 at the chronically acidified East Branch Neversink. Concentrations of Al_{im} averaged 0.006 and 0.017 mg/L at Black Brook

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Fig. 2. Calculated and measured inorganic monomeric aluminum (Al_{im}) concentrations, stream discharge, stream pH, and cumulative brook trout mortality observed during toxicity tests at (A) Black Brook, (B) High Falls Brook, (C) Biscuit Brook, and (D) the East Branch Neversink River, 1989–1990. Most graphs depict more than one toxicity test per season; start and end dates are in Table 2.

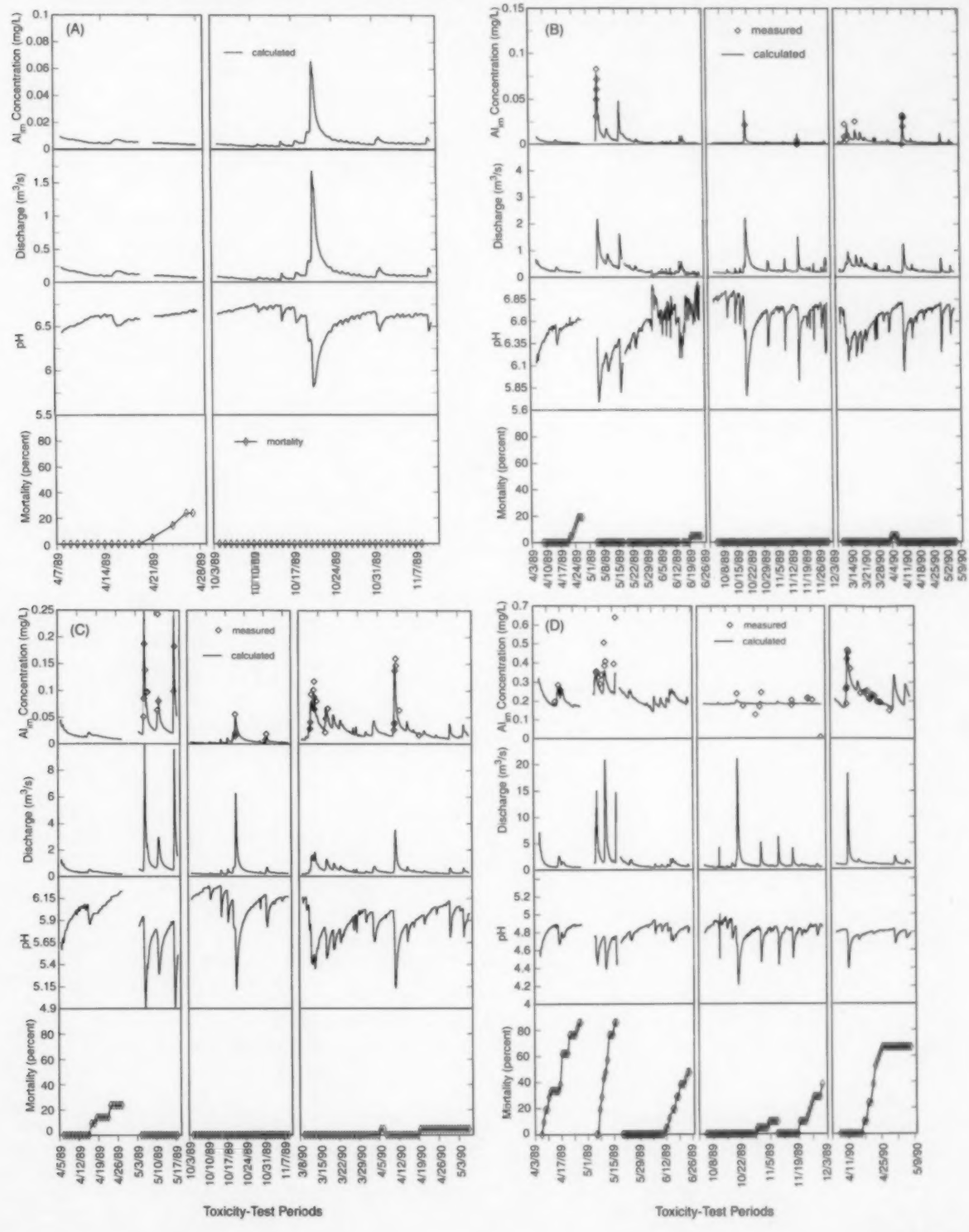

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Table 2. Test periods, percent mortality, and statistics for selected constituents in samples obtained from the four study streams during test periods.

Test dates		Percent	Calculated Al_{im}		Measured Al_{im}		DOC	pH	Mean	Mean
Start	End	mortality	Mean	Median	Mean	Median	Mean	Mean	Ca^{2+}	Cl^-
East Branch Neversink River										
4/6/89	4/27/89	81	0.208	0.193	0.248	0.253	3.19	4.83	1.5	0.638
5/5/89	5/16/89	86	0.279	0.266	0.398	0.369	3.84	4.57	1.23	0.54
5/19/89	6/24/89	48	0.204	0.199	na	na	3.63	4.7	1.71	0.469
10/4/89	11/7/89	10	0.184	0.183	0.189	0.172	4.92	4.68	1.26	0.599
11/8/89	11/30/89	38	0.184	0.183	0.201	0.204	3.15	4.77	1.4	0.594
4/6/90	5/6/90	67	0.227	0.211	0.272	0.243	2.78	4.69	1.4	0.544
Biscuit Brook										
4/6/89	4/27/89	24	0.015	0.014	na	na	1.87	5.99	2.87	0.683
5/4/89	5/17/89	0	0.048	0.031	0.099	0.096	3.16	5.35	2.44	0.573
10/4/89	11/8/89	0	0.005	0.003	0.023	0.018	3.20	5.97	2.66	0.686
3/9/90	4/6/90	5	0.030	0.027	0.071	0.074	2.32	5.64	2.91	0.542
4/6/90	5/6/90	5	0.022	0.018	0.083	0.063	2.42	5.64	2.79	0.55
High Falls Brook										
4/6/89	4/27/89	19	0.003	0.002	na	na	2.03	6.28	3.97	0.673
5/4/89	5/17/89	0	0.013	0.009	0.059	0.06	3.20	5.99	3.24	0.601
5/19/89	6/24/89	5	0.002	0.001	na	na	2.12	6.54	3.85	0.547
10/4/89	11/8/89	0	0.002	0.001	0.021	0.021	4.12	6.34	3.72	0.688
11/8/89	11/30/89	0	0.002	0.001	0.001	0	3.20	6.4	3.79	0.64
3/9/90	4/6/90	5	0.005	0.004	0.015	0.015	2.11	6.42	3.79	0.536
4/6/90	5/6/90	0	0.003	0.002	0.022	0.029	2.65	6.32	3.67	0.534
Black Brook										
4/8/89	4/27/89	24	0.005	0.004	na	na	1.57	6.51	4.53	0.661
10/4/89	11/9/89	0	0.006	0.004	0.072	0.072	3.48	6.35	3.99	0.621

Note: Number of values, *n*, used for each statistic ranged from 5 to 40. All chemical concentrations (except pH) are in mg/L. na, not available.

and High Falls Brook, 0.077 mg/L at Biscuit Brook, and 0.211 mg/L at the East Branch Neversink.

Most high flows were accompanied by pronounced fluctuations in pH, ANC, and concentrations of Al_{td}, Al_{tm}, Al_{im}, DOC, NO_3^-, SO_4^{2-}, Cl^-, and Ca^{2+} in the two acidified streams (East Branch Neversink and Biscuit Brook) and by smaller fluctuations in the two reference streams (High Falls Brook and Black Brook). Hourly discharge, calculated pH, and measured and calculated Al_{im} concentrations for each stream during toxicity-test periods (Figs. 2A–2D) suggest that pH and Al_{im} concentrations are directly related to discharge in the four streams, but the relations differ among streams and change seasonally within streams. For example, discharge in the East Branch Neversink increased about 20-fold during the middle of October 1989 and both measured and estimated concentrations of Al_{im} did not change significantly; however, similar increases in discharge during the spring of 1989 and 1990 were accompanied by large increases in Al_{im} concentration of as much as 0.500 mg/L (Fig. 2D). Seasonal changes in the relation between stream discharge and Al_{im} concentrations at Biscuit Brook (Fig. 2C) and at High Falls Brook (Fig. 2B) are also evident, although to a lesser degree. Conversely, fluctuations in stream pH consistently reflected changes in discharge at all sites; therefore, the relation between stream pH and discharge did not change greatly from season to season within any of the four streams.

Brook trout mortality

Brook trout mortality in the four streams ranged from 0 to 86% at the end of each test, and differed by stream and season (Table 2). Generally, fewer than 25% of brook trout died in spring tests (1989, 1990) in the three least acidified streams (Black Brook, High Falls Brook, and Biscuit Brook), whereas 48–86% of fish died in spring tests in the chronically acidified stream (East Branch Neversink) (Table 2). Mortality levels were lower in fall tests than spring tests at all sites (Figs. 2A–2D); during the fall of 1989, mortality was 0 at Black Brook, High Falls Brook, and Biscuit Brook, and 10–38% at the East Branch Neversink River. Mortality levels at the end of test periods typically correlated closely to mean and median pH and Al_{im} concentrations that fish were exposed to during the test period (Table 2).

Relations between brook trout mortality and chemical constituents

Results of simple regression analyses show that either median or mean Al_{im} concentrations alone could account for 76–85% of the variability in brook trout mortality (Table 3). The largest r^2 values and smallest SDRs generally resulted when mean or median Al_{im} concentration was used as the single independent variable; SDRs were 13–16% (eqs. 1–3 in Table 3). The statistics for other constituents typically had weaker relations with mortality levels (Table 3). Mean and median estimates of Al_{td}, Ca^{2+}, and pH could account for 40–65% of the variability in mortality (eqs. 6–11 in Table 3). Chloride and DOC were not significantly related to mortality (Table 3) but were included in stepwise multiple-regression analyses because they can alter either water toxicity or the sensitivity of fish to toxic condition (Schofield and Trojnar 1980; Baker and Schofield 1982; Driscoll 1985; Parkhurst 1987).

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Table 3. Slope, intercept, standard deviation of residuals (SDR), r^2 or adjusted r^2 value, p value, and degrees of freedom (df) for simple, logistic, and multiple regression equations defining relations between percent brook trout mortality (y) and statistics from measured constituents (x) during tests in the four study streams, 1989–1990.

Equation	Variables (x)[a]	Slope	Intercept	SDR	r^2	p	df
1	Median Al_{im}	267.2	–10.3	0.013	0.85	≤0.0001	13
2	Mean Al_{im}	248.5	–9.6	0.013	0.84	≤0.0001	13
3	Maximum Al_{im}	154.8	–7.0	0.016	0.76	≤0.0001	13
4	LCR mean Al_{im}	na	na	0.005	0.98	<0.001	14
5	LCR median Al_{im}	na	na	0.003	0.99	<0.001	14
6	Mean Al_{td}	206.7	–7.7	0.019	0.65	≤0.0001	13
7	Mean pH	–27.7	178.6	0.020	0.52	<0.001	18
8	Minimum pH	–29.5	174.8	0.022	0.40	0.003	18
9	Mean Ca^{2+}	–17.6	70.7	0.021	0.46	0.001	18
10	Mean DOC	4.1	8.7	0.029	0.02	0.609	18
11	Mean Cl^-	–91.5	75.4	0.028	0.04	0.387	18
12	Mean Al_{im}	264.9	16.0	0.012	0.85	≤0.0001	12
	Mean DOC	–8.6				0.094	
13	Median Al_{im}	284.1	14.9	0.012	0.87	≤0.0001	12
	Mean DOC	–8.5				0.080	
14	Median Al_{im}	386.6	–273.8	0.012	0.87	0.0001	
	Mean pH	57.2				0.088	11
	Mean Ca^{2+}	–26.4				0.178	
15	Median Al_{im}	325.0	–70.3	0.009	0.92	≤0.0001	11
	Mean DOC	–16.9				0.011	
	Mean Cl^-	181.3				0.003	
16	Mean Al_{im}	377.2	–156.5	0.008	0.93	≤0.0001	10
	Mean DOC	–17.7				0.002	
	Mean Cl^-	201.5				0.006	
	Mean pH	12.3				0.127	
17	Median Al_{im}	393.2	–143.0	0.008	0.93	≤0.0001	10
	Mean DOC	–16.1				0.003	
	Mean pH	12.0				0.013	
	Mean Cl^-	173.0				0.011	
18	Mean Al_{im}	372.9	–246.9	0.007	0.95	≤0.0001	9
	Mean DOC	–16.1				0.002	
	Minimum pH	30.4				0.029	
	Mean Cl^-	237.6				0.001	
	Mean Ca^{2+}	–8.5				0.246	
19	Median Al_{im}	377.3	–195.6	0.008	0.94	≤0.0001	9
	Mean DOC	–14.9				0.005	
	Mean Cl^-	201.1				0.005	
	Minimum pH	23.0				0.118	
	Mean Ca^{2+}	–5.8				0.481	

Note: na, not applicable.

[a] LCR, logistic concentration response (equation factors are provided in text); DOC, dissolved organic carbon; Al_{im}, inorganic monomeric aluminum; Al_{td}, total dissolved aluminum.

The sigmoid-shaped Gaussian or logistic concentration response (LCR) function (Fig. 3),

$$y = a + b/(1 + (x/c)^d),$$

generally describes mortality and toxic-constituent associations more accurately than linear equations (Rand and Petrocelli 1985). LCR equations that use mean or median measured Al_{im} data (x) account for 98–99% of the variability in mortality levels (y), and SDR values are as low as 3% (eqs. 4 and 5 in Table 3). Concentrations of other constituents did not generate practical LCR functions when related to our brook trout mortality data. Results indicate that percent mortality of brook trout (y) in Catskill tests is most accurately described when x is mean Al_{im} concentration, in milligrams per litre, and factors a, b, c, and d are equal to 78.03, 76.52, 0.20, and 32.54, respectively (Fig. 3A).

The two-, three-, four-, and five-variable regression equations that most accurately define brook trout mortality include median or mean Al_{im} concentration as the primary variable (eqs. 12–19 in Table 3). The linear associations of one or two constituent coefficients (t values) are not statistically significant in several equations but are included because the F value remains significant, and inclusion of the constituent typically improves the SDR by at least 1%. The two most accurate two-variable equations (eqs. 12 and 13) consist of mean or median Al_{im} and mean DOC (SDR = 12%, r^2 = 0.85 and 0.87). The

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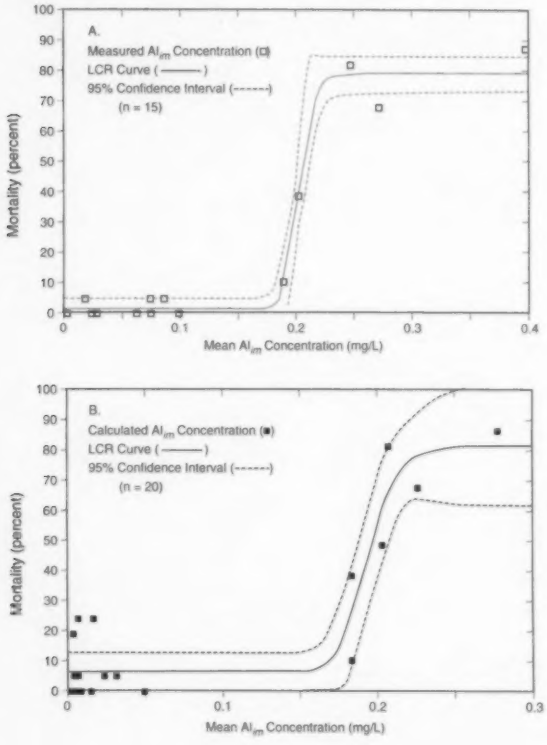
Fig. 3. Logistic concentration response (LCR) curve (solid lines) and 95% confidence interval (broken lines) describing the relations between percent brook trout mortality and mean inorganic monomeric aluminum (Al_{im}) concentration. (A) Relations based on measured Al_{im} concentrations. (B) Relations based on calculated hourly Al_{im} concentrations.

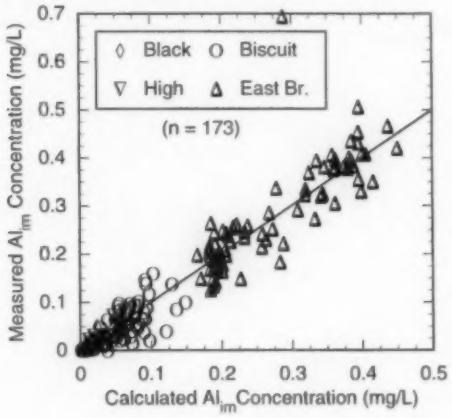


best three-variable equation (eq. 15) includes median Al_{im}, mean DOC, and mean Cl^- (SDR = 9%, $r^2 = 0.92$). A second three-variable equation (eq. 14), nearly as accurate as the two-variable equations, includes median Al_{im}, mean pH, and mean Ca^{2+} (SDR = 12%, $r^2 = 0.87$). Two four-variable equations (eqs. 16 and 17) include median or mean Al_{im} and mean DOC, mean Cl^-, and mean pH (SDR = 8%, $r^2 = 0.93$). The best five-variable equation (eq. 18) includes median Al_{im}, mean DOC, mean Cl^-, mean Ca^{2+}, and minimum pH (SDR = 7%, $r^2 = 0.95$). The second five-variable equation (eq. 19) is almost as accurate as eq. 18; it differs only in its use of mean Al_{im} rather than median Al_{im} (SDR = 8%, $r^2 = 0.94$).

Relations between brook trout mortality and calculated Al_{im} data

Hourly calculated Al_{im} concentrations represent measured Al_{im} concentrations fairly accurately and also depict temporal fluctuations in Al_{im} concentration for each stream. Although some Al_{im} measurements differ from the calculated Al_{im} record, most of the differences are small (Figs. 2A–2D). The accuracy of equations (not shown) used to calculate hourly Al_{im} concentrations is demonstrated by the relatively small SDR (0.033 mg/L) of the relation between all measured and calculated Al_{im} values from the four streams (Fig. 4). The

Fig. 4. Calculated and measured inorganic monomeric aluminum (Al_{im}) concentrations from all toxicity-test periods in the four Catskill Mountain streams, 1989–1990.



SDRs for relations between measured and calculated Al_{im} values are 0.018 mg/L ($r^2 = 0.53$) for Black Brook, 0.013 mg/L ($r^2 = 0.75$) for High Falls Brook, 0.031 mg/L ($r^2 = 0.49$) for Biscuit Brook, and 0.039 mg/L ($r^2 = 0.83$) for East Branch Neversink River. These SDRs are comparable to the total analytical error of approximately 0.024 mg/L for Al_{im} measurements in the laboratory.

Magnitude statistics (mean, median, and maximum values) for calculated Al_{im} concentrations account for less variability in percent mortality ($r^2 = 0.62$–0.74; eqs. 1–3 in Table 4) than do equivalent statistics for measured Al_{im} concentrations ($r^2 = 0.76$–0.80; eqs. 1–3 in Table 3); however, the SDR values of 15–18% are analogous. Even though statistics for measured Al_{im} data (Table 3, Fig. 3A) explain variability in mortality better than those for calculated Al_{im} data (Table 4, Fig. 3B), hourly calculated Al_{im} concentrations represent exposure conditions more completely than the former. Out of all the magnitude statistics for calculated Al_{im} data, the mean Al_{im} concentration explains the largest amount of variability in brook trout mortality (74%) and yields an SDR of 15% (eq. 1 in Table 4). The median calculated Al_{im} concentration (SDR = 15%, $r^2 = 0.73$) describes percent mortality nearly as well (eq. 2 in Table 4). The LCR equation that uses mean calculated Al_{im} concentrations (eq. 4 in Table 4) accounts for 83% of the variability in trout mortality (SDR = 11%).

Linear relations between brook trout mortality and the cumulative duration (hours) of exposure estimated above calculated-Al_{im} concentrations from 0.025 to 0.350 mg/L were significant; however, the slopes, r^2 values, and significance levels (p) decreased, and SDR values increased, in all equations in which Al_{im} concentrations were above and below the 0.225–0.250 mg/L range (Table 4). The relations between percent mortality and cumulative duration of exposure to Al_{im} concentrations of 0.225 and 0.250 mg/L (and higher) yielded the smallest SDR values (14 and 15%) and largest r^2 values (0.73 and 0.76) (eqs. 7 and 8 in Table 4). These results suggest that Al_{im} concentrations of 0.225–0.250 mg/L are pivotal to brook trout mortality.

Relations between percent mortality and the number (frequency) of acidic–Al_{im} episodes during which Al_{im} concentrations

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Table 4. Slope, intercept, standard deviation of residuals (SDR), r^2 or adjusted r^2 value, p value, and degrees of freedom (df) for simple and logistic regression equations defining the relations between percent brook trout mortality (y) and statistics of magnitude, duration, and frequency (x) for calculated Al_{im} concentrations (in milligrams per litre) in tests at the four Catskill Mountain streams, New York, 1989–1990.

Equation	Variable (x)	Slope	Intercept	SDR	r^2	p	df
	Magnitude statistics for calculated Al_{im} concentrations						
1	Mean concentration	249.8	2.8	0.015	0.74	≤0.0001	18
2	Median concentration	256.2	3.5	0.015	0.73	≤0.0001	18
3	Maximum concentration	149.7	–2.5	0.018	0.62	≤0.0001	18
4	LCR mean concentration	na	na	0.011	0.83	<0.001	18
	Duration of exposure (number of hours) above Al_{im} concentration threshold in column 2						
5	0.175 mg/L	0.058	10.1	0.023	0.36	0.003	18
6	0.200 mg/L	0.117	11.2	0.019	0.56	≤0.0001	18
7	0.225 mg/L	0.236	9.2	0.015	0.73	≤0.0001	18
8	0.250 mg/L	0.388	9.9	0.014	0.76	≤0.0001	18
9	0.275 mg/L	0.626	12.3	0.017	0.64	≤0.0001	18
10	0.300 mg/L	0.784	13.9	0.019	0.53	<0.001	18
11	0.325 mg/L	1.048	14.8	0.021	0.46	<0.001	18
	Frequency of exposure (number) to increases in Al_{im} concentration of at least the value in column 2						
12	0.050 mg/L	16.7	6.6	0.022	0.42	0.002	18
13	0.100 mg/L	20.6	7.5	0.021	0.46	0.001	18
14	0.200 mg/L	35.9	11.9	0.024	0.32	0.010	18

Note: Al_{im}, inorganic monomeric aluminum; LCR, logistic concentration response (equation factors are provided in the text); na, not applicable.

increased by 0.050, 0.100, 0.150, and 0.200 mg/L were significant; however, the SDR values were high, and r^2 values were low (eqs. 12–14 in Table 4). The lowest SDR, 21%, and highest r^2 value, 0.46, were obtained from the regression between percent mortality and the number of episodes in which Al_{im} concentrations increased by at least 0.100 mg/L.

The 10, 20, 50, and 100% brook trout mortality estimates (Fig. 5), as a function of exposure duration and Al_{im} concentration (eqs. 5–11 in Table 4 and others), suggest that certain Al_{im} magnitude and duration characteristics are important to brook trout mortality. The sharp decrease in slope of the 20 and 50% mortality lines at and below 0.200 mg/L Al_{im} provides evidence that this threshold is critical to brook trout mortality. The minimum period of exposure needed to cause death of caged brook trout in infrequently toxic waters of the Neversink River can also be hypothesized by comparing significant (20%) predicted mortality levels with the duration of exposure to Al_{im} concentrations in excess of the threshold for survival (0.225–0.250 mg/L). The 20% mortality line in Fig. 5 indicates that (*i*) only background mortality levels (defined as less than 20%) are expected in waters with mean Al_{im} concentrations below 0.175 mg/L for extended periods, and (*ii*) significant mortality levels (defined as 20% or greater) are expected in waters with mean Al_{im} concentrations of 0.225 mg/L (and higher) only when exposure durations are 2 days or longer.

Discussion

Our results demonstrate that waters of three low-order Catskill Mountain streams, Biscuit Brook, High Falls Brook, and the East Branch, were episodically acidified during events of increased discharge; the East Branch Neversink River was also chronically acidified. Discharge events on the three streams typically produced concurrent episodes of elevated Al_{im} concentration; the frequency, duration, and magnitude of increases in Al_{im} concentration depended upon stream and season. Al_{im} concentrations were often toxic to caged young-of-the-year brook trout in the East Branch site and seldom toxic to caged fish at the other two acidified sites or at the primary reference site, Black Brook. With the single exception of Van Sickle et al. (1996), no other studies have documented fish mortality in the Catskills or associated it with acidification of local streams.

In our study, brook trout mortality was related to mean and median Al_{im} concentration more strongly than to other constituents. Mean pH and DOC, Ca^{2+}, and Cl^- concentrations were also significantly related to brook trout mortality. Acidic–Al_{im} episodes appeared to cause significant brook trout mortality when Al_{im} concentrations approached or exceeded 0.225 mg/L but only after fish were exposed to these conditions for 2 or more days. Except for the ERP (the present study; Gagen et al. 1993; Simonin et al. 1993; Van Sickle et al. 1996), few studies have assessed the relations between fish mortality and fluctuating concentrations of Al_{im} in natural systems. Many recent investigations, however, have found that temporal characteristics of fluctuating pH and Al_{im} concentrations (in the laboratory) and mean and median pH, Ca^{2+}, DOC, and Al_{im} concentrations (in both the laboratory and natural systems) strongly affect fish survival and are good predictors of brook trout mortality (see review by Baker et al. 1990).

Biologically relevant chemical constituents

Calcium

Our finding that mean Ca^{2+} concentrations can account for 46% of the variability in brook trout mortality is consistent with results of previous studies. Calcium has been shown to improve brook trout survival by increasing their tolerance to Al_{im} in (*i*) pH-neutral waters at Ca^{2+} concentrations above 0.5–1.0 mg/L, and (*ii*) acidic waters at Ca^{2+} concentrations of

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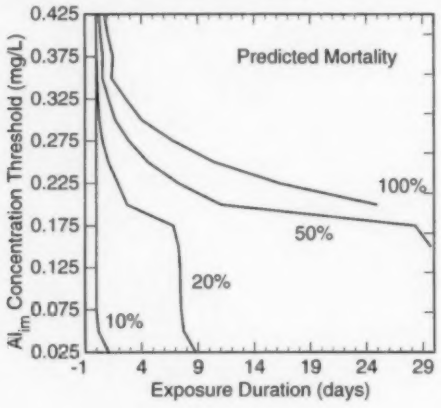
Fig. 5. Estimated 10, 20, 50, and 100% brook trout mortality as a function of exposure duration (0–30 days) and inorganic monomeric aluminum (Al_{im}) concentration (0.025–0.425 mg/L).



2.0–8.0 mg/L (see review by Baker et al. 1990). For example, during exposure of brook trout fry to toxic levels of Al and acidity, whole-body content of Na, Cl^-, Ca^{2+}, and K^+ in brook trout fry declined (susceptibility to ionoregulatory distress increased) except when Ca^{2+} concentrations exceeded 1.5–2.0 mg/L (Wood et al. 1990*a*, 1990*b*). The authors concluded that (*i*) a protective Ca^{2+} threshold exists for brook trout fry, (*ii*) it ranges from 2 to 8 mg/L, and (*iii*) the protective threshold varies with pH. Calcium increases the tolerance of fish to otherwise toxic levels of Al_{im} and acidity by increasing the efficiency of sodium ion regulation and oxygen exchange processes, which are impeded by low pH and high Al_{im} concentrations (Mount et al. 1988).

In our study, mean Ca^{2+} concentrations were less than 2.0 mg/L during tests in the East Branch Neversink River where pH averaged less than 5.0, and generally higher than 2.0 mg/L in the other three streams where pH averaged 5.4–6.5. Evidently, tolerance levels and survival rates of exposed fish were not positively affected by high Ca^{2+} concentrations at the East Branch Neversink site because of very low pH levels; high mean Ca^{2+} concentrations at the other sites may have provided some protection for brook trout exposed to high Al_{im} concentrations.

Chloride

In Catskill tests, mean Cl^- concentrations alone were not significantly related to percent mortality, but multiple regression analyses (eqs. 15–19 in Table 3) suggested that Cl^- was possibly the fourth or fifth most important constituent affecting brook trout mortality. These findings advocate a hypothesis that, during exposure to toxic acid and Al_{im} concentrations, elevated concentrations of Cl^- (and Ca^{2+}, Na^+, and K^+) in stream waters decrease the osmotic imbalance between fish and water (the NaCl gradient) and thereby reduce ionoregulatory stresses, sensitivity of fish, and mortality rates in a manner similar to that of Ca^{2+}. Circumstantial evidence from the literature suggests that high Cl^- concentrations may, in fact, decrease the sensitivity of brook trout to Al_{im} and pH toxicity. Several studies document a decrease in whole-body content of Cl^- (and Na^+, Ca^{2+}, and K^+) in salmonids during exposure to elevated acidity and Al concentrations (Wood et al. 1990*a*, 1990*b*; Cleveland et al. 1991; Gagen and Sharpe 1987; Gagen et al. 1993). None, however, have directly related increased fish survival with elevated Cl^- concentrations in acidified lakes and streams. Uniformly low concentrations of Cl^- in our study streams (0.5–0.7 mg/L; Tables 1 and 3) likely lessen the strength of any interrelations and, thus, the importance of Cl^- to brook trout mortality in our analyses.

Dissolved organic carbon

The higher levels of fish mortality and concentrations of Al_{tm} and Al_{im} during the spring than the fall (Fig. 2, Table 2), coupled with higher concentrations of DOC and Al_{om} during the fall than the spring (Table 2), suggest that seasonal differences in brook trout mortality in Catskill streams are due, in part, to seasonal differences in DOC concentrations. During the fall, high concentrations of DOC complex inorganic Al species, which in turn increases Al_{om} concentrations. During the spring, both DOC and Al_{om} concentrations are low. Although primarily controlled by pH, mean Al_{im} concentrations during the spring are much higher than they are during the fall at most study sites (Table 2); Al_{im} concentrations also increase more sharply during spring episodes of high flow and increased acidity than during comparable fall episodes (Fig. 2). In the three Catskill streams with measurable Al_{im} concentrations, shifts in the ratios of Al_{om} to Al_{tm} between seasons demonstrate a potential seasonal effect of DOC on Al_{im} concentration and on water toxicity. Mean ratios of Al_{om} to Al_{tm} at High Falls Brook, Biscuit Brook, and the East Branch Neversink were 1.00, 0.80, and 0.41 during the fall and 0.69, 0.46, and 0.18 during spring tests, respectively. The larger fraction of Al_{tm} that is Al_{im} during the spring as compared with the fall makes the waters potentially more toxic to aquatic life during the spring even at equivalent fall pH levels and Al_{tm} concentrations. The seasonal difference in Al_{im} concentration and water toxicity in these three streams may therefore be attributed indirectly to DOC concentration and the strong direct relation between Al_{om} and DOC concentrations ($r^2 = 0.50, p \leq 0.0001$). Driscoll et al. (1980) and Shultz et al. (1993) described similar relations between Al_{om} and DOC concentrations, and higher levels of both during fall than during spring in Adirondack and Catskill waters.

Given the strong association between DOC, Al_{om}, and Al_{im} and the seasonal differences in mortality levels, DOC, Al_{om}, and Al_{im} concentrations, it is surprising that DOC alone was not significantly related to brook trout mortality in the Catskill tests (Table 3). DOC was, however, found to be the second most important factor in all multiple-variable regressions (eqs. 12–19 in Table 3); thus, our findings were consistent with other studies that generally indicate that DOC is a strong secondary or tertiary determinant of brook trout mortality (Parkhurst et al. 1990; Simonin et al. 1993). Numerous studies confirm that DOC complexes with inorganic Al to create Al_{om} species that are less toxic than Al_{im} to fish (Driscoll et al. 1980; Driscoll 1985; Parkhurst 1987). Others have shown that concentrations of Al_{om}, like Al_{im}, are systematically dependent on pH (Browne and Driscoll 1993). Results from these and other investigations also suggest that, in moderately acidified waters, DOC concentrations greater than 2.0 or 3.0 mg/L may significantly decrease Al_{im} concentrations and, thus, decrease water toxicity and mortality of exposed brook trout. For example,

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Parkhurst (1987) and Parkhurst et al. (1990) showed that, for their studies, (*i*) the protective effects of DOC were most pronounced when the DOC concentration was greater than 3.1 mg/L and water pH was 5.2–5.6, (*ii*) very high Al_{im} concentrations (1.0 mg/L) inhibited the protective effects of DOC, (*iii*) DOC was the third most important factor related to brook trout mortality, and (*iv*) a logistic model that included Al_{im}, pH, and DOC best predicted brook trout survival in waters with DOC concentrations greater than 1.0 mg/L.

In our analyses, the relation between brook trout mortality and DOC alone was only partially evident because any protective effect of high DOC concentrations (>2.0 mg/L) at the East Branch Neversink site was negated, as predicted by Parkhurst et al. (1990), by high Al_{im} concentrations (up to 0.639 mg/L) and very low pH values (4.6–4.8) (Table 1). Mean DOC concentrations at this site ranged from 2.8 to 3.8 mg/L during five tests where brook trout mortality exceeded 20% and averaged 4.9 mg/L in one test with mortality less than 20% (Table 2). Mean DOC concentrations in the well-buffered stream (Black Brook) and the two intermediate-pH streams (Biscuit Brook and High Falls Brook) were generally less than 2.0 mg/L when brook trout mortality was equal to or greater than 20% and were 2.0–4.1 mg/L when mortality was less than 20% (Table 2). Brook trout mortality and DOC concentrations were also not significantly related to each other during toxicity tests done in four Adirondack streams, yet median DOC was the second most important factor in multiple-variable analyses, accounting for an additional 17% of variability in mortality (Simonin et al. 1993). During all of their tests, median DOC ranged from 2.9 to 9.3 mg/L (Simonin et al. 1993). DOC concentrations in many Adirondack waters are typically high (>3.0 mg/L) and Al_{om} is usually the dominant Al form (Driscoll et al. 1980). Conversely, seasonal differences in brook trout mortality levels in several Pennsylvania streams were attributed to higher acidity and higher Al_{td} concentrations during the spring than in the fall; DOC was unimportant to brook trout survival because more than 90% of Al_{td} was found to be in a reactive inorganic form (Al_{im}) year round (Gagen et al. 1993). Except for Benner Run during the fall 1989, consistently low DOC concentrations, averaging from about 1 to 2 mg/L during spring tests and from 2 to 3 mg/L during fall tests, may have lessened the effect of DOC on Al speciation and fish mortality in their streams (Gagen et al. 1993). In a statistical analysis of chemistry and bioassay results from all of the ERP streams, Van Sickle et al. (1996) found that median DOC concentrations added explanatory power to higher order brook trout mortality models using time-weighted median Al_{im}, minimum pH, and median Ca^{2+}.

Findings of this study, Gagen et al. (1993), Simonin et al. (1993), and Van Sickle et al. (1996) suggest that the beneficial effect of DOC on brook trout mortality varies across the northeastern United States owing to differences in DOC concentrations encountered in streams of the regions. These differences may be enhanced, and the importance of DOC on fish mortality further altered, by complex geochemical interactions that underlie relations among pH, DOC, Al_{om}, and Al_{im}. Spatial (local and regional) and temporal differences in DOC concentrations and in the effect of DOC on Al_{im} concentration and fish mortality may be attributed to three factors: (*i*) Al_{im} and DOC can be important pH buffers in acidic waters (pH <5.3), where the buffering capacity of dissolved inorganic carbon is diminished, (*ii*) high DOC concentrations can contribute to water acidity and toxicity, and (*iii*) the surface-water concentration of Al_{im} is highly related to pH while Al_{om} concentration is highly related to DOC concentration (Driscoll 1985; Browne and Driscoll 1993).

pH

Our analyses indicate that pH is a secondary determinant of brook trout mortality in the Catskill study streams. Mean pH alone accounted for 52% of the variability in mortality, and including pH in equations with three or more predictor variables (Table 3) helped account for an additional 11% of the variability. These results corroborate findings of earlier studies that indicate that pH is often a primary or secondary determinant of fish mortality in lakes and streams of the northeastern United States that undergo acidification (Driscoll et al. 1980; Baker and Schofield 1982; Sharpe et al. 1987; Johnson et al. 1987; Parkhurst et al. 1990; Gagen et al. 1993; Van Sickle et al. 1996). Other investigations have also shown that acidity is acutely toxic to various life stages of brook trout in waters with a pH range of about 3.5–4.5 and that it is a nonlethal inhibitor of growth at moderate levels (pH 5.0–6.0) (see review by Baker et al. 1990). In our study streams, however, pH rarely approached acutely toxic levels for young-of-the-year brook trout, even in the chronically acidic East Branch Neversink site (Table 2, Fig. 2).

The effect of pH on brook trout mortality in the Catskill tests appears to be due primarily to its relation to mineral Al solubility. Analyses of Catskill stream-chemistry data through a simple chemistry equilibrium model, ALCHEMI (Schecher and Driscoll 1987), indicate that Al solubility is controlled by an aluminum hydroxide solid phase similar to gibbsite. Water pH controls dissolution of gibbsite and, therefore, also controls Al speciation and Al_{im} concentration (Driscoll 1985). Dissolution of gibbsite increases both above and below pH 6.0; thus, concentrations of inorganic Al species increase as pH decreases below pH 6.0 (Driscoll 1985). Because water pH strongly affects the concentration of Al_{im}, and because Al_{im} appears to govern brook trout mortality in the Catskill tests, pH is critical to brook trout mortality and to the distribution of native brook trout populations in Catskill streams that are affected by acidification.

Inorganic monomeric aluminum

Our results indicate that Al_{im} is the primary determinant of brook trout mortality in the study streams and that significant brook trout mortality occurs only after fish are exposed to Al_{im} concentrations in excess of a survival threshold for 2 days or longer. In fact, mean or median Al_{im} concentration accounted for 76–99% of the variability in brook trout mortality (Table 3). Recent investigations have also concluded that Al_{im} was the primary determinant (and that pH and DOC were significant secondary determinants) of mortality of brook trout in natural acidified waters (see review by Baker et al. 1990; Parkhurst et al. 1990). Mean or median Al_{im} (or Al_{td}) concentration or a combined duration and Al_{im} concentration factor consistently explained the greatest amount of variability in brook trout mortality in all three regions of the ERP (Gagen et al. 1993; Simonin et al. 1993; Van Sickle et al. 1996).

In our study streams, 0.200 mg/L Al_{im} could be designated as the concentration threshold for brook trout survival given

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that (*i*) 20% represents the maximum acceptable brook trout mortality level (based on the total error of mortality data; see Methods), (*ii*) the estimated 20% lethal Al_{im} concentration (LC_{20}) for brook trout is in the 0.181–0.195 mg/L range (LCR curves in Figs. 3A and 3B), and (*iii*) duration of exposure to Al_{im} concentrations in excess of 0.225 and 0.250 mg/L accounts for the greatest amount of variability in mortality (Table 4). This finding is consistent with recent field and laboratory studies that have documented significant brook trout mortality under low Ca^{2+} (<2.0 mg/L), DOC (<2.0 mg/L), and pH (4.4–5.2) conditions only when ambient concentrations of Al_{im} and (or) Al_{id} exceed the 0.200–0.300 mg/L range (see review by Baker et al. 1990; Van Sickle et al. 1996). For example, gill damage and mortality in brook trout increased at pH values of 4.2–5.2 only when total acid-soluble Al concentrations were equal to or greater than 0.200 mg/L (Schofield and Trojnar 1980; Baker and Schofield 1982); brook trout mortality occurred only when they were exposed (for some minimum duration) to Al_{id} concentrations greater than 0.200–0.300 mg/L (Gagen and Sharpe 1987).

Duration and frequency of acidic–aluminum episodes

Results from the Catskill study provide evidence that acidic–Al_{im} episodes strongly reduce survival of resident brook trout in parts of the Neversink River. Increased mortality was generally associated only with periods of high flow and episodes of elevated acidity and increased Al_{im} concentration (Figs. 2A–2D). Results also indicate that certain episode characteristics affect brook trout mortality more than others. In linear regression analyses, for example, episode frequency appears to have a minor effect on mortality, whereas the cumulative duration of exposure to Al_{im} concentrations in excess of 0.250 mg/L explained the greatest amount of variability (76%) in brook trout mortality (Table 4). Assuming that the Al_{im} concentration threshold for survival is about 0.200 mg/L (Figs. 3 and 5), the 20% mortality line in Fig. 5 shows that significant brook trout mortality is projected at 0.225 mg/L Al_{im} only when exposure durations are 2 days or longer. This critical minimum exposure is supported by physiological data from Gagen and Sharpe (1987), which show that ionoregulation fails and brook trout die at toxic Al_{id} concentrations (above the 0.200–0.300 mg/L range) only when exposure durations are 1.5 days or longer.

Mounting evidence from other field and laboratory investigations also suggests that the frequency of acidic–Al_{im} episodes and the duration of exposure to lethal and sublethal conditions strongly affect mortality levels. Death of brook trout occurs only after a minimum exposure period to toxic Al_{id} concentrations is surpassed, apparently when whole-body content and depletion rates of Na^+ and other ions surpass a minimum physiological threshold for survival (Gagen and Sharpe 1987). A combined Al_{im} concentration and exposure-duration variable was the best single predictor of brook trout mortality in bioassays done in the Adirondack Mountains (Simonin et al. 1993). Repeated exposure to hydrogen ion pulses of varying durations (acid episodes) caused greater cumulative mortality of larval brook trout than continuous exposures to equivalent mean hydrogen ion concentrations (Curtis et al. 1989). Intermittently elevated Al_{id} concentrations caused greater cumulative mortality and decreased growth rates of juvenile brook trout than continuous exposures to similar concentrations (Siddens et al. 1986). These findings and the effect of episode number and duration of exposure to toxic Al_{im} concentration on brook trout mortality in Catskill tests suggest that (*i*) the frequency of toxic conditions can significantly affect brook trout survival, (*ii*) stresses and injury to brook trout exposed to toxic acidic–Al_{im} episodes may be additive, (*iii*) brook trout appear to undergo little recovery between frequent toxic acidic–Al_{im} episodes in headwater streams, and (*iv*) brook trout appear to assimilate effects of acidic–Al_{im} episodes through the cumulative damage–repair function described by Breck (1988).

The total exposure duration and the frequency of exposure to elevated concentrations of toxic constituents can be as important to fish survival as the ambient concentration of the toxicant. The duration of exposure affects mortality levels because stress and death of organisms are generally not provoked at low concentration of toxins during long exposures nor at high concentrations during extremely short exposures (Breck 1988); therefore, the duration of exposure can be assumed to determine the ultimate level of mortality for exposed fish once Al_{im} concentrations exceed the threshold for survival. The number or frequency of exposures to toxic conditions can also affect mortality rates if the resulting injury to an organism is additive and not repaired between successive sublethal exposures (Rand and Petrocelli 1985; Breck 1988). Thus, knowledge of the maximum duration that fish can survive acutely toxic Al_{im} concentrations (the minimum duration that causes mortality during exposure to Al_{im} concentrations at and above survival thresholds), and of the effects of frequent changes in Al_{im} concentration on fish mortality, is essential to assess the significance of acidic–Al_{im} episodes on individual fish. The effects of chronic acidification and acidic–Al_{im} episodes on mortality of one the most acid-tolerant native species (brook trout) suggest that endemic fish populations and fish communities throughout the Neversink River are also negatively affected by stream acidification.

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Bootstrap confidence limits for groundfish trawl survey estimates of mean abundance

Stephen J. Smith

Abstract: Trawl surveys using stratified random designs are widely used on the east coast of North America to monitor groundfish populations. Statistical quantities estimated from these surveys are derived via a randomization basis and do not require that a probability model be postulated for the data. However, the large sample properties of these estimates may not be appropriate for the small sample sizes and skewed data characteristic of bottom trawl surveys. In this paper, three bootstrap resampling strategies that incorporate complex sampling designs are used to explore the properties of estimates for small sample situations. A new form for the bias-corrected and accelerated confidence intervals is introduced for stratified random surveys. Simulation results indicate that the bias-corrected and accelerated confidence limits may overcorrect for the trawl survey data and that percentile limits were closer to the expected values. Nonparametric density estimates were used to investigate the effects of unusually large catches of fish on the bootstrap estimates and confidence intervals. Bootstrap variance estimates decreased as increasingly smoother distributions were assumed for the observations in the stratum with the large catch. Lower confidence limits generally increased with increasing smoothness but the upper bound depended upon assumptions about the shape of the distribution.

Résumé : Les relevés au chalut selon des plans d'échantillonnage aléatoire stratifié sont couramment utilisés sur la côte est de l'Amérique du Nord pour surveiller les populations de poisson de fond. Les estimations sont calculées par randomisation et ne nécessitent pas l'établissement d'un modèle probabiliste pour les données. Toutefois, les propriétés de ces estimations, qui correspondent à des échantillons de grande taille, ne conviennent pas nécessairement aux échantillons de petite taille ni aux données asymétriques caractéristiques des relevés au chalut de fond. Dans notre étude, nous avons employé trois méthodes de rééchantillonnage bootstrap intégrant des plans d'échantillonnage complexes pour explorer les propriétés des estimations correspondant à de petits échantillons. Nous présentons une nouvelle formule permettant de calculer les intervalles de confiance après correction du biais et accélération dans les relevés à échantillonnage aléatoire stratifié. Les résultats de simulation indiquent que les limites des intervalles de confiance après correction du biais et accélération peuvent surcorriger les données des relevés au chalut, et que les limites en percentiles étaient plus proches des valeurs prévues. Des estimations non paramétriques de la densité ont servi à étudier les effets de captures anormalement grandes de poisson sur les estimations bootstrap et les intervalles de confiance correspondants. Plus les distributions supposées pour les observations situées dans la strate des fortes captures étaient lisses, plus les estimations bootstrap des variances étaient faibles. Les limites de confiance inférieures montaient généralement en même temps qu'augmentait le degré de lissage, mais la limite supérieure dépendait des hypothèses sur la forme de la distribution.
[Traduit par la Rédaction]

Introduction

Annual bottom trawl surveys provide a major source of fisheries independent information on abundance, species composition, and basic biological data for the groundfish communities of the Atlantic coast of Canada and the United States (Azarovitz 1981; Halliday and Koeller 1981; Pitt 1981). Most of these surveys use a stratified random design with stratum boundaries defined by depth ranges, species-specific distributions, and management areas. The abundance estimates of fish from these surveys are routinely used in the stock assessment of many commercial groundfish species (Smith 1988*a*; Gunderson 1993).

The statistical properties of quantities commonly estimated from stratified random trawl surveys such as mean and total catch are usually estimated using standard methods from sample survey or finite population theory (Smith 1990). Standard error estimates are derived assuming repeated sampling from the finite population (e.g., trawl sites). Confidence intervals are constructed on the basis of the normal distribution, which was shown to be the limiting distribution for the stratified mean (and total) when the central limit theorem was applied to sampling from a finite population (Cochran 1977; Thompson 1992). However, in many cases, small sample sizes within strata and observed fish catches exhibiting skewed frequency distributions result in confidence limits that are not very useful. In particular these confidence limits may have extremely long intervals between them or even negative lower limits on occasion.

Alternatively, a number of statistical models for positive random variables (e.g., Poisson, Δ distribution) have been suggested as possible models for catch per tow (Taylor 1953; Pennington 1983; Smith 1988*b*; McConnaughey and Conquest 1993). The usual advantages given for using such models include (*i*) the provision of more precise estimates of the mean and (*ii*) the possibility (but not necessarily the means) of obtaining confidence intervals that are always positive. However, Smith (1990) has shown how the application of statistical models and their specific estimates to survey data can result in

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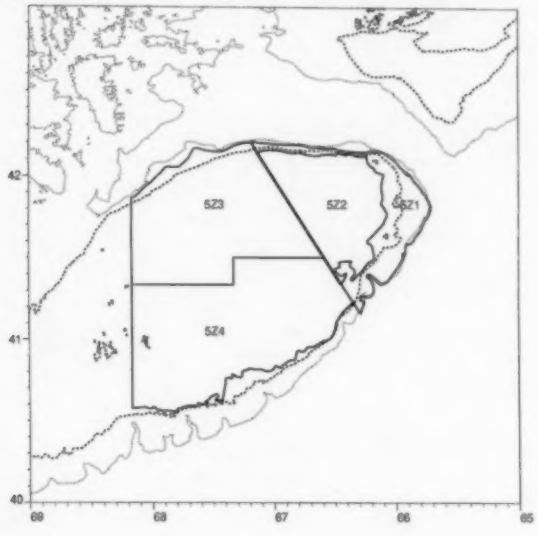
Fig. 1. Stratification map for Georges Bank surveys (1987–present). Stratum boundaries are based primarily on the average spatial distribution in February–March of Atlantic cod and haddock. The numbers on the map identify the individual strata.

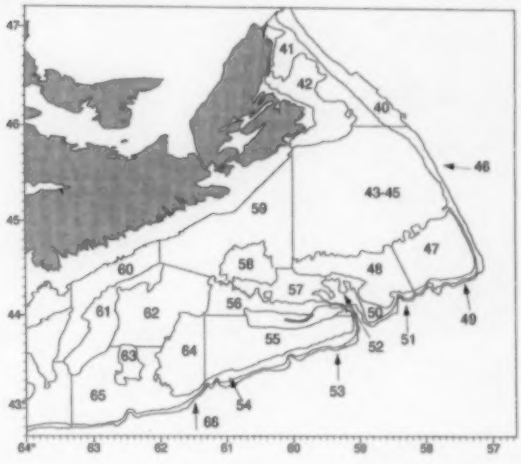


biased estimates of the population means and variances normally estimated in sample survey theory (see also Jolly and Smith 1989; Skinner et al. 1989; Myers and Pepin 1990).

A third approach for modelling the distribution of estimates from surveys is to use bootstrap resampling methods (Efron 1982). Bootstrap methods have been used in a number of fisheries survey applications (e.g., Kimura and Balsinger 1985; Sigler and Fujioka 1988; Robotham and Castillo 1990; Pelletier and Gros 1991; Buckland et al. 1992; Stanley 1992; Smith and Gavaris 1993) as a means of substituting computational power for theoretical analysis in situations requiring complex models or estimates. The bootstrap offers a natural way of modelling survey estimates given that its basis is very similar to that of the randomization basis for finite population theory. Bootstrap confidence intervals do not require a distributional assumption for their construction and thus can be used to evaluate the standard normal theory intervals.

In this paper, I apply the bootstrap methodology to the construction of confidence intervals for the stratified mean number of haddock (*Melanogrammus aeglefinus*) per tow from groundfish trawl surveys of the Scotian Shelf and Georges Bank. The application of the bootstrap methodology to complex survey designs is not straightforward and three published methods for stratified survey designs are compared here. A number of methods exist for estimating bootstrap confidence intervals and three of these methods are compared. A new form for the bias-corrected and accelerated bootstrap confidence interval is presented for stratified survey designs. Nonparametric density estimates are used to evaluate these bootstrap confidence intervals. Finally, nonparametric density estimates are also used to investigate the impact on bootstrap estimates of different hypotheses concerning the frequency distribution of catch data when the occasional extremely large catch of fish is encountered.

Fig. 2. Stratification map for the Scotian Shelf surveys conducted in July (1970–present). Stratum boundaries are based primarily on depth ranges (originally measured in fathoms). The numbers on the map identify the individual strata.



Materials and methods

Trawl survey data

The fish catch data sets used in this study were obtained from the standard groundfish trawl surveys of Georges Bank and the Scotian Shelf conducted by the Marine Fish Division (Bedford Institute of Oceanography, Dartmouth, N.S., and Biological Station, St. Andrews, N.B.) of the Canadian Department of Fisheries and Oceans. These surveys use a stratified random survey design (Cochran 1977; Smith 1988*a*). The Georges Bank strata (Fig. 1) were based on the historical distribution of Atlantic cod (*Gadus morhua*) and haddock during February–March, and in addition, incorporated the Canada–U.S. boundary (Gavaris and Eeckhaute 1990). The eastern Scotian Shelf strata (Fig. 2) were primarily based on depth boundaries of 91, 183, and 366 m (originally 50, 100, and 200 fathoms). Further delineations of the stratum boundaries reflect species and stock distributions (Doubleday 1981; Halliday and Koeller 1981).

The sample unit for the survey is defined as the area over the bottom covered by a trawl of a specific width (12.5 m) towed at 3.5 knots (1 knot = 1.852 km/h) for a distance of 1.75 nautical miles (1 nautical mile = 1.852 km). The positions of these sample units or sets were selected randomly before the cruise for each stratum. The design, operation, and analysis of stratified random trawl surveys on the Scotian Shelf have been discussed by Halliday and Koeller (1981) and Smith (1988*a*). We will need the following definitions for quantities associated with the trawl surveys in any one year: n_h is the number of hauls or sets sampled in stratum h ($h = 1, \ldots, L$); $n = \Sigma_{h=1}^{L} n_h$, the total number of sets sampled in the survey; N_h is the total number of possible sets in stratum h; $N = \Sigma_{h=1}^{L} N_h$, the total number of possible sets in the survey area; $f_h = n_h/N_h$, the sampling fraction in stratum h; $W_h = N_h/N$, the proportion of the area in stratum h; y_{hi} is the number of fish caught in set i and stratum h; $\bar{y}_h = \Sigma_{i=1}^{n_h} y_{hi}/n_h$, the estimated mean abundance in stratum h; and $s_h^2 = \Sigma_{i=1}^{n_h} (y_{hi} - \bar{y}_h)^2/(n_h - 1)$, the estimated variance in stratum h.

The stratified mean abundance and its associated variance are estimated as

$$(1) \qquad \bar{y}_{st} = \sum_{h=1}^{L} W_h \bar{y}_h$$

and

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$$(2)\qquad \widehat{\mathrm{Var}}(\bar{y}_{st}) = \sum_{h=1}^{L} \frac{N_h}{N^2}(N_h - n_h)\frac{s_h^2}{n_h}$$

respectively.

There are two important aspects of eq. 2 that may not be readily apparent from the formula. In the first place, the variance that is being measured here concerns the effectiveness of estimating the mean catch over all N_h units, $\bar{Y}_h$, with the sample mean, that is, the expected value over all strata of $(\bar{y}_h - \bar{Y}_h)^2$ for all distinct samples of size n_h repeatedly chosen from the N_h possible sample units within each stratum. This formulation is unaffected by any spatial structure that may exist for the species being captured, although temporal stability of the fish distribution is implicitly assumed. Secondly, this estimate gives the variance of the sample mean as a predictor of what may be expected to be caught in those sites where trawls were not made (see, for example, Smith 1990). The more sample units that are observed, the better the sample mean will be as a predictor of fish catch.

Parametric confidence intervals for the stratified mean are constructed by assuming that under repeated sampling the $\bar{y}_{st}$ have a normal or Student's t distribution (Cochran 1977, pp. 95–96). The effective degrees of freedom for the Student's t are estimated as

$$\mathrm{df}_e = \frac{\left(\sum_{h=1}^{L} g_h s_h^2\right)^2}{\sum_{h=1}^{L} \frac{g_h^2 s_h^4}{n_h - 1}}$$

where $g_h = N_h(N_h - n_h)/n_h$. This method is valid even if the variances differ between strata. However, the method does require that the y_{hi} are normally distributed so that the individual terms of the sum in the denominator are estimates of the variance of s_h^2.

Bootstrap methods for stratified random designs

The bootstrap resampling method has been successfully used to provide standard error estimates and nonparametric confidence intervals for both simple linear statistics and nonsmooth functions of data (Efron 1982). The basic idea of the bootstrap is to treat the original sample of size n as the target population and the original estimated statistic (e.g., mean, ratio) as the population parameter to be estimated. Repeated sampling with replacement of size n from the original data set is used to create a large number of new pseudosamples. Estimates of the parameter of interest are made for each of the pseudosamples and the empirical distribution of these resultant estimates is used to characterize the distribution of the original statistic. Bias is evaluated with respect to the difference between the average of all of the bootstrap estimates and the original estimate. In cases where it is known that the original statistic and its variance are unbiased, the bootstrap estimate and its variance estimate must also be so.

The bootstrap method was originally introduced for independently and identically distributed cases where all sample units had an equal probability of being chosen. In complex survey designs, such as stratified random designs, the sample units have the same probability of being chosen within any one stratum but different strata may have very different sampling intensities. Following are three different approaches of extending the bootstrap method to sample survey estimates from complex survey designs.

Naïve bootstrap

The simplest (and hence the most naïve) approach to applying the bootstrap to samples from a stratified random design is to resample the observations independently within each stratum, that is,

1. Within stratum h take a simple random sample of the y_{hi} of size n_h with replacement and calculate $\bar{y}_h^*$. Repeat independently for all L strata.

2. Calculate the stratified mean as $\bar{y}_{st,b}^* = \sum W_h \bar{y}_h^*$.

3. Repeat steps 1 and 2 a total of B times to get $\bar{y}_{st,1}, \ldots, \bar{y}_{st,B}$.

The bootstrap estimate of the stratified mean is calculated as

$$(3)\qquad \bar{y}_{st}^* = \sum_{b=1}^{B} \bar{y}_{st,b}^*/B$$

with variance given by

$$(4)\qquad \widehat{\mathrm{Var}}(\bar{y}_{st}^*) = \frac{1}{B-1}\sum_{b=1}^{B}(\bar{y}_{st,b}^* - \bar{y}_{st}^*)^2$$

Given that the observed stratified mean (eq. 1) and variance (eq. 2) are unbiased estimates we would expect the expected values of $\bar{y}_{st}^*$ and $\mathrm{Var}(\bar{y}_{st}^*)$ to equal their respective observed values. While the bootstrap estimate of the mean is an unbiased estimate of the stratified mean, the expectation of the bootstrap variance has been shown to be equal to (Efron 1982; Rao and Wu 1988)

$$(5)\qquad E(\widehat{\mathrm{Var}}(\bar{y}_{st}^*)) = \sum_{h=1}^{L} W_h^2\left(\frac{n_h - 1}{n_h}\right)\frac{s_h^2}{n_h}$$

and therefore the naïve bootstrap variance is an inconsistent estimate of the variance in eq. 2. While a correction could be derived to obtain a consistent estimate of the variance, this would be missing the point, because the purpose here is to generate a population of bootstrap observations with the expected mean and variance.

Rescaling bootstrap

Rao and Wu (1988) proposed rescaling the resampled values of resample size m_h ($m_h \leq n_h$) so that the resulting bootstrap variance estimate would be equal to its expected value in eq. 2. Determination of the appropriate resample sizes is the key to finding the most consistent estimates of the variance. The method of Rao and Wu (1988) proceeds as follows:

1. Take a simple random sample of size m_h ($\leq n_h$) with replacement from the given sample in each stratum h (y_{hi}^*, $i = 1, \ldots, m_h$). This is done independently for each stratum. Calculate

$$\tilde{y}_{hi} = \bar{y}_h + \left(\frac{m_h(1 - f_h)}{(n_h - 1)}\right)^{1/2}(y_{hi}^* - \bar{y}_h)$$

$$\tilde{y}_h = m_h^{-1}\sum_{i=1}^{m_h}\tilde{y}_{hi}$$

$$\tilde{y}_{st} = \sum_{h=1}^{L} W_h \tilde{y}_h$$

2. Replicate step 1 B times to obtain $\tilde{y}_{st,1}, \ldots, \tilde{y}_{st,B}$.

The bootstrap estimates of the stratified mean and variance are calculated in exactly the same way as for the naïve bootstrap by substituting $\tilde{y}_{st,b}$ for $\bar{y}_{st,b}^*$ in eqs. 3 and 4.

Rao and Wu (1988) suggested setting $m_h = n_h - 3$, on the basis of comparing the bootstrap third moment $E_*(\bar{y}_{st,b} - \tilde{y}_{st})^3$ with the unbiased estimate of the third moment of $\bar{y}_{st}$. In a recent study, Kovar et al. (1988) compared the merits of setting $m_h = n_h - 3$ to $m_h = n_h - 1$ and found that their results favoured the latter over the former. Smith and Gavaris (1993) reported similar results for a limited example. However, we will only be able to compare $m_h = n_h - 3$ with $m_h = n_h - 1$ for the Georges Bank example because a number of the n_h in the Scotian Shelf survey were less than or equal to 3.

While the rescaling method has been found to provide more unbiased estimates of the variance than the naïve method, it has the disadvantage of being a more computer-intensive method.

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Table 1. Summary of quantities used in calculating the stratified mean and variance for the number of haddock caught in the 1989 Georges Bank survey.

Stratum (h)	n_h	W_h	$\bar{y}_h$	s_h
5Z1	18	0.1622	27.82	23.90
5Z2	26	0.2555	85.94	120.99
5Z3	8	0.3069	2.19	5.78
5Z4	6	0.2755	1.05	1.74

Note: The sample size, mean, and standard deviation for each stratum are given by n_h, $\bar{y}_h$, and s_h, respectively. The proportion of the total survey area in each stratum is given in the column headed by W_h.

Mirror-match bootstrap

Sitter (1992*a*) designed the mirror-match bootstrap to imitate the original within-stratum sampling scheme and provide unbiased estimates of the stratified variance.

1. Take a simple random sample without replacement of size m_h ($\leq n_h$) from stratum h to obtain a new set of observations $y'_{h1}, \ldots, y'_{h,m_h}$.
2. Repeat step 1 k_h times independently, replacing the resamples of size m_h each time to get k_h groups of observations.
3. Calculate the mean for stratum h as $\bar{y}'_h = \sum_{l=1}^{k_h}\sum_{j=1}^{m_h} y'_{hjl}/(k_h m_h)$, where l and j refer to the observations chosen in the without- and with-replacement steps.
4. Repeat steps 1 and 2 for each stratum and calculate the stratified mean as per eq. 1, replacing $\bar{y}_h$ with $\bar{y}'_h$.
5. Replicate step 1 B times to obtain $\bar{y}'_{st,1}, \ldots, \bar{y}'_{st,B}$.

As in the previous case, the bootstrap estimates of the stratified mean and variance are calculated in exactly the same way as for the naïve bootstrap by substituting $\bar{y}'_{st,b}$ for $\bar{y}^*_{st,b}$ in eqs. 3 and 4.

In the first step m_h is chosen such that the sampling fraction m_h/n_h is as close as possible to that for the original sample n_h/N_h. This step is repeated k_h times, where $n_h = k_h m_h$ so that the final sample size is the same as the original sample size. However, if $n_h/N_h \leq 1/n_h$ then the replication factor in step 2 is calculated as $k_h = n_h(1 - m_h/n_h)/m_h(1 - n_h/N_h)$. The sample size in step 1 is obtained as $m_h = n_h(1 - m_h/n_h)/(1 - n_h/N_h)$. If either k_h or m_h is noninteger, a randomization between bracketing integers is used.

The sampling fractions n_h/N_h for the data in this study are all <0.0003 whereas the smallest $1/n_h$ is equal to 0.038 and therefore m_h defaults to 1.0. The replication factor k_h will be a noninteger bracketed by $n_h - 1$ and n_h. Following Sitter (1992*a*) an integer value k'_h is chosen by using a Bernoulli random number generator,

$$k'_h = \begin{cases} n_h - 1 & \text{when random number} = 1 \\ n_h & \text{when random number} = 0 \end{cases}$$

where the probability of obtaining a random number = 1 is

$$P(x=1) = \frac{\left(\frac{(1-f_h)}{(n_h-1)} - \frac{1}{n_h}\right)}{\left(\frac{1}{n_h-1} - \frac{1}{n_h}\right)}$$

This special case of the mirror-match method (i.e., $m_h = 1$) is equivalent to the bootstrap with replacement (BWR) method of McCarthy and Snowden (cited in Sitter 1992*a*). This method is also similar to the naïve method with the addition of a randomization step for choosing either n_h or $n_h - 1$ resamples within each stratum.

Confidence intervals from bootstrap resampling

There are a number of ways of computing percentiles for confidence intervals from bootstrap estimates (see Efron and Tibshirani 1993). The three considered here, the percentile method (PC), the bias-corrected method (BC), and the bias-corrected and accelerated method (BC_a), are the more commonly used methods. The PC method assumes that the frequency distribution of the bootstrap estimates fully describes the distribution function of some estimate $\hat{\theta}$, $G(s) = \text{Prob}(\hat{\theta} \leq s)$. That is,

$$\hat{G}(s) = \sum_{i=1}^{B} I_i / B$$

where

$$I_i = \begin{cases} 1, & \text{if } \hat{\theta}^*_i \leq s; \\ 0, & \text{otherwise.} \end{cases}$$

and $\hat{\theta}^*_i$ denotes the ith bootstrap estimate of θ (e.g., stratified mean) and B as before ($i = 1, \ldots, B$) denotes the number of bootstrap replications. Upper and lower α confidence intervals are calculated as $\hat{G}^{-1}(1-\alpha/2)$, $\hat{G}^{-1}(\alpha/2)$, respectively.

The BC method (Efron 1981) introduces a correction to the PC method to account for differences between $\hat{\theta}$ and the median of the frequency distribution. The α upper and lower confidence intervals for this bias-corrected method are obtained as $\hat{G}^{-1}(\Phi(z^{(1-\alpha/2)} + 2z_0))$, $\hat{G}^{-1}(\Phi(z^{(\alpha/2)} + 2z_0))$, respectively; where Φ is the standard normal distribution function, z^t is the tth percentile of the standard normal distribution, and

$$z_0 = \Phi^{-1}\left(\hat{G}^{-1}\left(\frac{\#(\hat{\theta}^*_i < \hat{\theta})}{B}\right)\right)$$

(where #( ) refers to a count of how many times the condition within the parentheses is true). The term z_0 will be equal to zero when the bootstrap estimate and the median of the $\hat{\theta}^*_i$ are equal; the bias-corrected and percentile methods are equivalent in this case.

Finally, the standard normal approximation often used in constructing confidence intervals assumes that the mean is independent of the variance. However, in many cases and certainly for trawl survey catch data this assumption does not hold. A further correction factor, a, referred to as the acceleration is introduced as a measure of the rate of change of the standard error of $\hat{\theta}$ with respect to the true parameter value θ measured on a normalized scale. The α upper and lower confidence intervals for the BC_a method are given as

$$\hat{G}^{-1}\left(\Phi\left(z_0 + \frac{z_0 + z^{(1-\alpha/2)}}{1 - \hat{a}(z_0 + z^{(1-\alpha/2)})}\right)\right)$$

and

$$\hat{G}^{-1}\left(\Phi\left(z_0 + \frac{z_0 + z^{(\alpha/2)}}{1 - \hat{a}(z_0 + z^{(\alpha/2)})}\right)\right),$$

respectively.

Note that setting both z_0 and $\hat{a}$ to zero would result in the formula for the percentile confidence intervals. The acceleration is estimated here using the jackknife-based estimate (Efron and Tibshirani 1993, p. 186) modified for stratified random surveys (see Appendix).

Results

Distribution of survey data

The data used in this paper are from the 1989 winter groundfish survey on the eastern portion of Georges Bank and the 1988 summer groundfish survey of the Scotian Shelf. The haddock catches from each survey are summarized in Tables 1 and 2. The abundance indices for the haddock stock on Georges Bank were estimated from catches in strata 5Z1–5Z4 (Fig. 1). The four strata were fairly similar in size (W_h) and enjoyed a higher sampling intensity per stratum than many of the surveys in the Northwest Atlantic. For the eastern Scotian Shelf haddock

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stock, abundance indices were estimated from catches in strata 40–66 (Fig. 2). This survey was more characteristic of the usual situation in the Northwest Atlantic (Doubleday 1981) by having many strata of highly variable size, with the number of sets per stratum ranging from 2 to 8 in most years.

The frequency distributions for both data sets exhibited highly skewed distributions with large proportions of zeros (greater than 40% of the observations) and long right-hand tails (Fig. 3). The empirical cumulative distribution functions were estimated using the standard estimator for stratified random designs given in Chambers and Dunstan (1986). Up to a point the distributions were similar for the two areas; however, there was one extremely large catch of 5496 haddock in the eastern Scotian Shelf survey. In fact, this was the largest catch of haddock in the history of this survey.

Bootstrap estimate of the variance of the stratified mean

Before proceeding to investigate the bootstrap distributions of the stratified mean, the variance estimates (eq. 4) from the three bootstrap methods, naïve, rescale, and BWR, were compared with the estimate from the usual stratified variance of the mean (eq. 2). The percent deviation of the various bootstrap estimates from the stratified variance estimate is presented as a function of the number of replications (B) in Fig. 4 for the Georges Bank data set and in Fig. 5 for the eastern Scotian Shelf. As advertised, the naïve method resulted in a biased estimate of the variance. All of the other methods gave reasonable estimates of the variance, with the BWR method having the minimum deviation for the Scotian Shelf data set and the rescale method with $m_h = n_h - 1$ performing better for the Georges Bank data set. However, differences between the rescale methods and the BWR method were quite minimal. The naïve method will be retained for the rest of this study for comparative purposes even though the variance estimates were biased.

Another aspect of the comparison in Figs. 4 and 5 is an appreciation for the number of bootstrap replications required to get stable variance estimates. Reference levels for bootstrap resample sizes have been given as 20–50 for estimating standard errors (Efron and Tibshirani 1993, p. 273); however, these results indicate that much higher levels are required when dealing with complex survey designs. Variance estimates for the rescale and BWR methods converged much more quickly to the true value for the Georges Bank data set ($B \approx 300$) than they did for the Scotian Shelf data ($B \approx 750$), possibly a reflection of the higher sampling intensity in the former data set.

Bootstrap distribution of stratified mean

The empirical distribution curves for 1000 bootstrap estimates of the stratified mean for each of the four bootstrap methods for the Georges Bank data (Fig. 6) were quite smooth and there appeared to be very little difference between the different bootstrap methods. On the other hand, the empirical cumulative distributions for the eastern Scotian Shelf data plotted in Fig. 7 showed a number of differences. The distributions for the rescale and BWR methods were quite similar whereas the naïve method deviated from the other two curves at the 35th percentile and higher. All of the curves showed a number of discontinuities in the curves where few or no bootstrap estimates of the stratified mean were obtained. These discontinuities were the result of a problem common to trawl surveys: one very large catch of fish in the survey. Recall that in Fig. 3, the maximum observation for the 4VW haddock survey was 5496 fish, which was a considerable catch compared with the next largest catch of 309 haddock. This next largest catch also occurred in the same stratum (56) as the maximum catch. This large catch of 5496 haddock was quite influential in that by itself it accounted for 49% of the stratified mean. In contrast, the largest catch in the Georges Bank survey (425) only accounted for 15% of its stratified mean.

Table 2. Summary of quantities used in calculating the stratified mean and variance for the number of haddock caught in the 1988 eastern Scotian Shelf survey.

Stratum (h)	n_h	W_h	$\bar{y}_h$	s_h
40	6	0.0294	0.34	0.84
41	4	0.0318	7.40	5.50
42	7	0.0457	0.77	0.86
43	4	0.0419	0.26	0.51
44	4	0.1247	1.29	2.57
45	4	0.0325	0.00	0.00
46	3	0.0156	0.00	0.00
47	6	0.0513	24.67	45.03
48	5	0.0460	0.00	0.00
49	2	0.0046	17.73	11.32
50	3	0.0122	75.72	85.38
51	2	0.0047	2.06	2.91
52	2	0.0110	55.70	77.40
53	2	0.0082	0.00	0.00
54	2	0.0159	45.80	44.40
55	7	0.0674	94.90	59.60
56	6	0.0303	985.90	2212.80
57	2	0.0258	18.00	25.50
58	3	0.0209	77.90	118.90
59	6	0.1000	24.90	48.80
60	3	0.0427	18.60	17.20
61	2	0.0367	0.00	0.00
62	4	0.0672	2.70	4.80
63	2	0.0096	89.70	26.30
64	5	0.0412	109.30	110.50
65	8	0.0757	63.90	65.60
66	2	0.0072	1.50	2.20

Note: The sample size, mean, and standard deviation for each stratum are given by n_h, $\bar{y}_h$, and s_h, respectively. The proportion of the total survey area in each stratum is given in the column headed by W_h.

The discontinuities in Fig. 7 separate segments of the curve, which going from the bottom to the top of the figure give the distribution of the stratified mean when the catch of 5496 haddock was not chosen or chosen once, twice, or three or more times for the bootstrap sample in its stratum. The fact that this one catch would occur more than once in a bootstrap sample is not too surprising given that there were only six sets taken in stratum 56 and resampling was done with replacement.

All of the salient quantities being estimated for both data sets are presented in Table 3. The estimates of the stratified mean for all methods were within less than 1% of their respective original observed values. The variance estimates in Table 3 verify that the expected values of the naïve variance estimate were well predicted by eq. 5 and that all of the other bootstrap estimates were quite close to their respective original observed variance estimates.

Confidence intervals calculated using the methods given in

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Fig. 3. Empirical cumulative distribution plots for numbers of haddock caught from each tow in eastern Scotian Shelf (1988) and Georges Bank bottom trawl surveys (1989). Note that the maximum observation for the eastern Scotian Shelf survey was 5496 haddock.

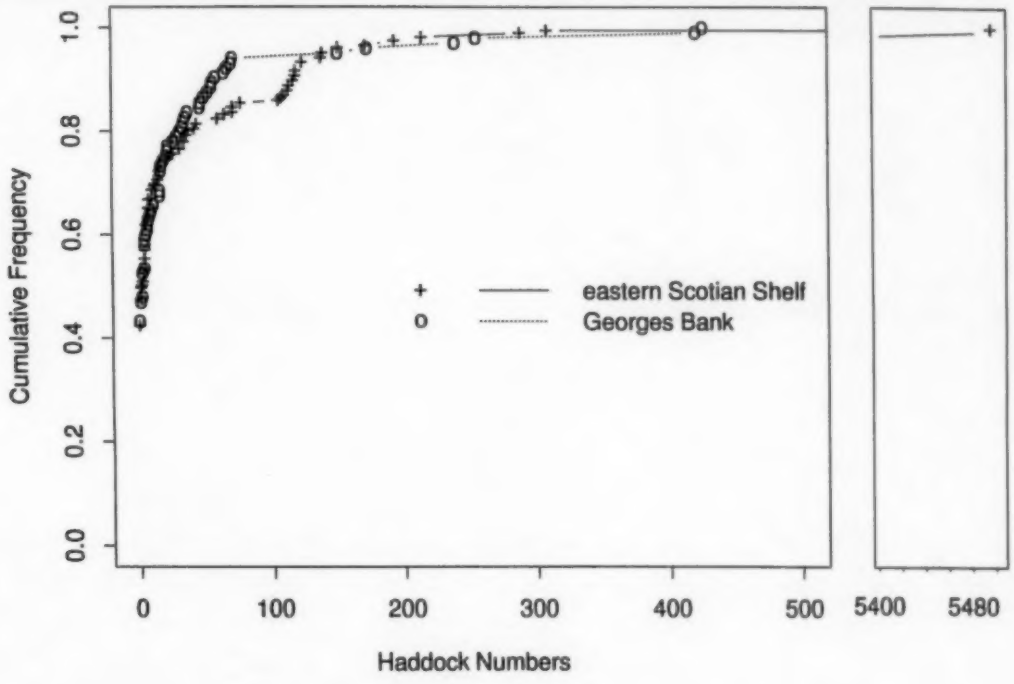

Fig. 4. Comparison of the performance of the variance estimates from each of four bootstrap methods presented here as a function of replication number. Performance was measured as the percent deviation of the bootstrap estimate from the original variance of the stratified estimate. Variance estimates are for the bootstrap stratified mean number of haddock per tow from the Georges Bank survey (1989).

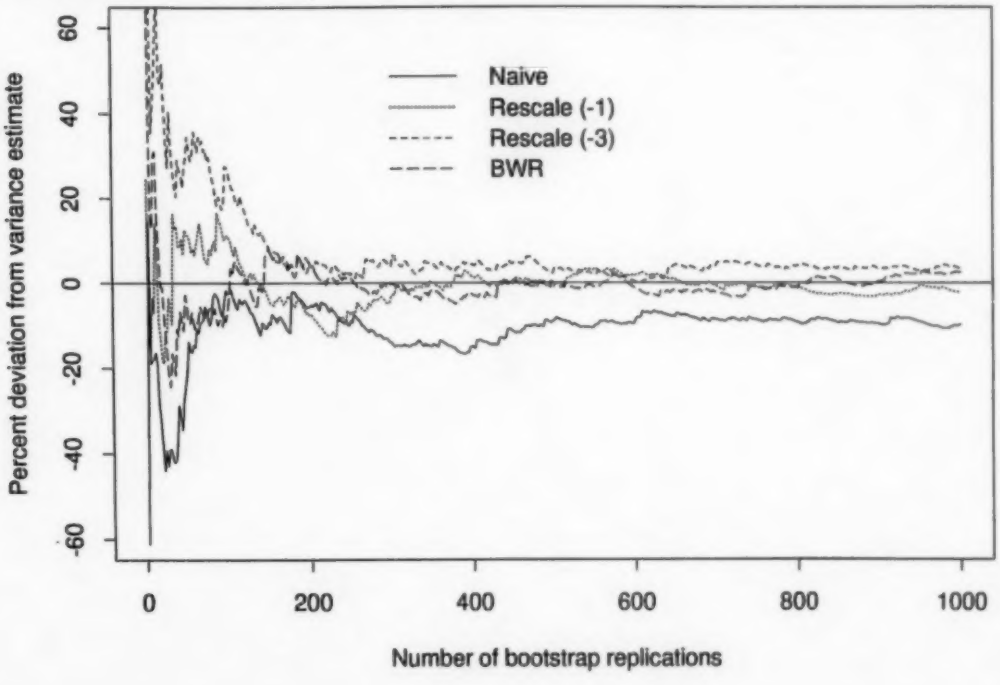

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Fig. 5. Comparison of the performance of the variance estimates from each of three bootstrap methods presented here as a function of replication number. Performance was measured as the percent deviation of the bootstrap estimate from the original variance of the stratified estimate. Variance estimates are for the bootstrap stratified mean number of haddock per tow from the eastern Scotian Shelf survey (1988).

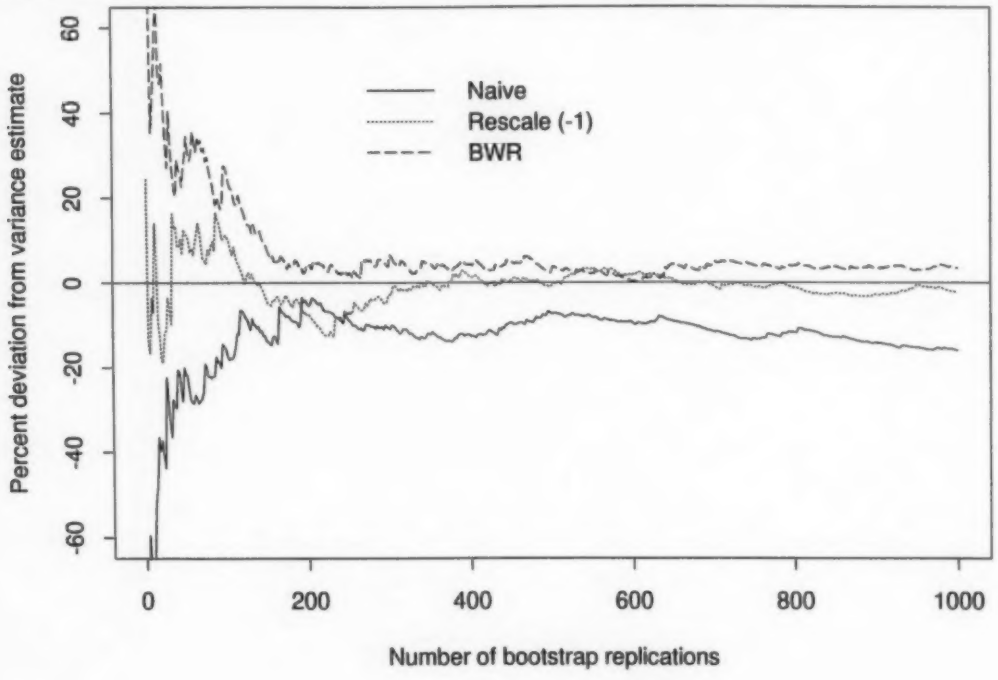

Fig. 6. Empirical cumulative distributions of the stratified mean number of haddock per tow from the Georges Bank survey (1989) estimated by four bootstrap methods.

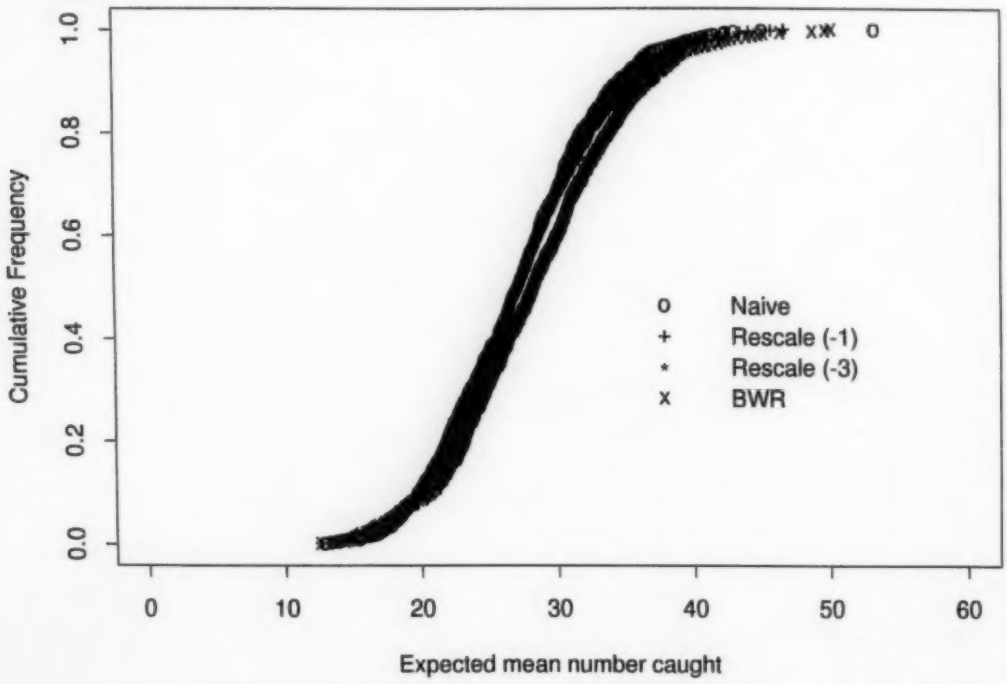

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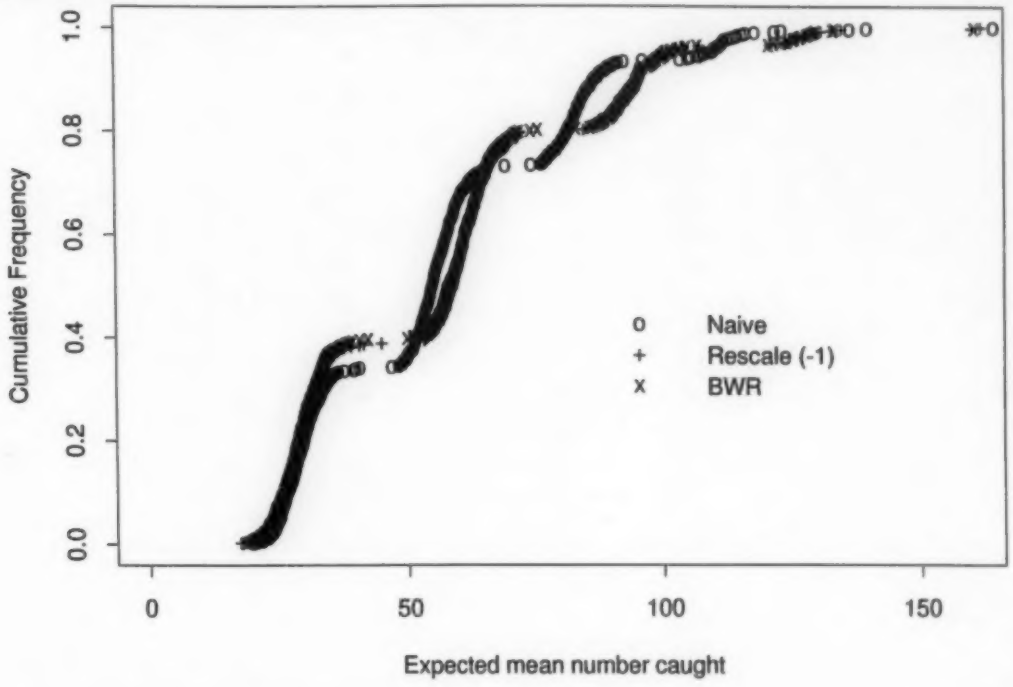
Fig. 7. Empirical cumulative distributions of the stratified mean number of haddock per tow from the eastern Scotian Shelf survey (1988) estimated by three bootstrap methods.



this paper are presented in Table 3 along with information on their length and shape. Shape is calculated as the natural log of the ratio of the upper limit minus the median to the median minus the lower limit (Efron 1992). Therefore, a confidence interval that is symmetric around the median will have a shape measure of zero whereas a shape greater than zero indicates distributions skewed to the right.

While the Student's t confidence intervals for the Georges Bank data set appear to be reasonable, the upper and lower bounds for the eastern Scotian Shelf haddock illustrate the problems with using the normal approximation. That is, the intervals can be quite long and the lower limit can be less than zero. The longer interval for the eastern Scotian Shelf survey reflects the small sample sizes within strata and the large variance from stratum 56 where the catch of 5496 haddock was made.

Citing general findings in the literature, Cochran (1977) suggested that $1 - \alpha$ confidence intervals for the mean based on the normal assumption will behave as follows when the original data have a skewed distribution. First, the area covered between the upper and lower limits will be less than $1 - \alpha$. Additionally, the probability of the mean being less than the lower limit will be less than $\alpha/2$ whereas the probability of it being greater than the upper limit will be greater than $\alpha/2$. If the bootstrap limits are to be an improvement then their respective upper and lower limits should be greater than those from the Student t distribution.

All of the bootstrap intervals resulted in positive lower limits that were all higher than those given by the Student t method. For the case of the eastern Scotian Shelf, these lower limits were substantially higher than the negative lower limit from the Student t. However, the bootstrap upper limits were not always greater than the respective Student t upper limits. Considering only the unbiased bootstrap methods, bootstrap upper and lower limits increased for each confidence interval method progressing from rescale with $m_b = n_b - 1$, $m_b = n_b - 3$ to the BWR method. Within any one bootstrap method, BC limits were greater or less than their respective PC limits depending upon whether or not the median was less or greater than the bootstrap estimate. In all cases the BC_a upper and lower limits were greater those of the other bootstrap methods and the Student t method. Within each bootstrap method the BC_a intervals were the longest and had the most skew, that is, the highest shape measure. The estimated acceleration was less for the Georges Bank data ($a = 0.06$) than for the eastern Scotian Shelf ($a = 0.12$), possibly reflecting the lower variability of the former relative to the latter.

Given that the naïve bootstrap underestimated the variance of the stratified mean it was expected that the corresponding confidence intervals would then be shorter than those for the other bootstrap methods. While this was true for all of the bootstrap methods and confidence interval methods for the Georges Bank data it was only true for the PC limits for the eastern Scotian Shelf. The larger correction for the median (acceleration) for the naïve bootstrap in this latter data set appeared to have compensated for the underestimation of the variance.

Coverage of confidence intervals

Given the observed data only, it is difficult to say how good the confidence intervals in Table 3 really were because there is no theoretical statistical distribution for survey catches or mean

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Table 3. Summary of results from calculating the stratified mean, median of the bootstrap distribution, variance, and 95% confidence intervals for the number of haddock caught in the 1989 Georges Bank survey and the 1988 eastern Scotian Shelf survey.

				95% confidence interval			
Method	Mean	Median	Variance	Type	Lower	Upper	Length	Shape
Georges Bank survey								
Original	27.43	*	38.00	St	14.77	40.08	25.31	0.00
Naïve bootstrap	27.16	27.17	34.27	PC	17.04	39.48	22.44	0.19
				BC	17.03	39.46	22.43	0.19
				BC_a	17.88	41.02	23.14	0.40
Rescale bootstrap								
$m_h = n_h - 1$	27.36	27.20	37.08	PC	15.70	39.28	23.58	0.05
				BC	16.15	39.70	23.55	0.06
				BC_a	17.26	41.49	24.23	0.30
$m_h = n_h - 3$	27.50	27.04	39.31	PC	16.49	40.02	23.53	0.21
				BC	17.05	40.98	23.93	0.20
				BC_a	18.08	44.09	26.01	0.52
BWR bootstrap	27.60	27.07	38.95	PC	16.82	40.93	24.11	0.30
				BC	17.56	41.67	24.11	0.26
				BC_a	18.55	43.97	25.42	0.52
Eastern Scotian Shelf survey								
Original	56.15	*	769.1	St	−14.20	126.50	140.70	0.00
Naïve bootstrap	56.19	54.89	646.4	PC	23.69	112.20	88.51	0.61
				BC	24.64	115.71	91.08	0.56
				BC_a	25.88	139.62	113.70	0.94
Rescale bootstrap								
$m_h = n_h - 1$	56.54	58.93	768.6	PC	21.94	124.82	102.88	0.58
				BC	20.35	99.84	79.49	1.40
				BC_a	22.26	127.12	104.90	1.87
BWR bootstrap	56.41	58.21	769.7	PC	22.36	125.46	103.10	0.63
				BC	20.96	102.79	81.84	1.09
				BC_a	22.83	128.28	105.40	1.45

Note: The confidence intervals for the original method were calculated assuming a Student's t distribution (St). Confidence intervals for the bootstrap mean were calculated using the percentile (PC), bias-corrected (BC), and bias-corrected accelerated (BC_a) methods. The length column refers to the length of the confidence intervals. The shape column refers to a measure of symmetry (symmetric: shape = 0) given by Efron (1992). Note that the expected values for the variances of the naïve bootstraps (eq. 5) for Georges Bank and the eastern Scotian Shelf were 36.59 and 640.30, respectively.

*By definition equivalent to the mean for the Student t distribution.

survey catches within a stratum. While the lower limits from the bootstrap methods tended to move in the right direction relative to the Student t, the bootstrap upper limits were not always greater than those from the normal approximation. Also, the limits themselves do not help evaluate whether or not the probabilities of being less than the lower or greater than the upper limit were actually 0.025 with 0.95 between the limits.

In lieu of not having a statistical model for these data, an alternative approach was taken here. Statistical distributions were fitted to the catches within each stratum of the Georges Bank data using nonparametric density functions (Silverman 1986; Scott 1992). In this approach, discrete kernel estimates were used (Rajagopalan and Lall 1995) with different bandwidths for each stratum. The theoretical observations ranged from zero to the maximum catch plus the bandwidth. The fitted distributions were assumed to represent a predictive distribution of what catches could be expected in each stratum (Jones and Bradbury 1993). Random observations were generated from the cumulative mass function for each stratum using the same number of samples per stratum as in Table 1. A total of 10 000 replications of the stratified mean were generated. The 0.025 and 0.975 limits were 16.64 and 40.39 (shape = 0.25), respectively, assuming that these 10 000 replications represent the population distribution for the stratified mean. The lower and upper limits for each method in Table 3 (Georges Bank data) were compared with the population distribution of the stratified mean. The actual proportions of observations in the population less than the lower and greater than the upper are given in Table 4. In addition, the proportions of observations between each upper and lower limit are also given in this table.

The Student t interval was wider than expected and the asymmetry in the actual distribution of the stratified mean shows that both upper and lower limits were too low, as was predicted for the normal approximation. The higher bootstrap lower limits result in the associated probabilities being closer to the expected value of 0.025 than for the Student t limit. Overall, the PC limits for the rescale bootstrap with $m_h = n_h - 3$ and the BWR bootstrap are closest to having 0.025 in the tails of all of the limits for all of the methods. For the Georges Bank data, the adjustments for the median and acceleration in the BC and BC_a limits appeared to have increased the confidence limits higher than they needed to be for the α level used here.

The lower than expected coverage for the naïve bootstrap reflects the previously noted underestimation of the stratified variance. The coverage was closest to 0.95 for the PC limits

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Table 4. Comparison of Student *t* and bootstrap confidence intervals with population confidence intervals constructed from 10 000 replications generated from nonparametric mass functions fitted to the Georges Bank data.

Method	Type	Lower	Upper	Coverage
Original	St	0.008	0.028	0.964
Naïve bootstrap	PC	0.030	0.033	0.937
	BC	0.029	0.034	0.937
	BC_a	0.045	0.020	0.934
Rescale bootstrap				
$m_h = n_h - 1$	PC	0.014	0.036	0.951
	BC	0.018	0.031	0.952
	BC_a	0.032	0.018	0.950
$m_h = n_h - 3$	PC	0.022	0.029	0.950
	BC	0.030	0.021	0.949
	BC_a	0.049	0.009	0.942
BWR bootstrap	PC	0.026	0.022	0.952
	BC	0.038	0.017	0.945
	BC_a	0.060	0.010	0.930

Note: Entries in lower and upper columns refer to the probabilities from the population curve relative to the lower and upper bound from each of the methods used herein. Coverage refers to the probability between the upper and lower limits. The expected probability is 0.025 each for upper and lower limits and the expected coverage is 0.95.

for the rescale and BWR bootstraps with the corrections by the BC_a method being far too severe, particularly for the BWR bootstrap interval.

These results are only directly applicable to the discrete nonparametric density estimate for Georges Bank data. There were too few observations within many of the strata of the Scotian Shelf survey to fit nonparametric densities.

Large catches

The normal approximation and the three types of bootstrap confidence limits were all affected by the large catch of 5496 haddock in the eastern Scotian Shelf survey. Such a large catch appears to be atypical when compared with the other observations in the same survey. From the finite population prediction point of view (see Smith 1990), the estimates of the mean and variance for stratum 56 assume that approximately one sixth of the area was represented by this large catch, an assumption we may believe to be unwarranted. The question of how representative the large catch was of other potential catches in the area could have been dealt with at the time of the survey by adding more fishing sets in the stratum in an adaptive sampling type approach (Thompson 1992). The larger number of sets per stratum in the Georges Bank survey may have protected this survey from being dominated by one large set. More observations in stratum 56 of the eastern Scotian Shelf survey could have decreased the weighting associated with the large catch if such large catches were less common than one out of six sets. However, this survey was completed in 1988 and so the point is moot.

On the other hand, it may be quite useful to be able to postulate various alternatives for the distribution of catches in this stratum and evaluate their effect on the bootstrap confidence intervals. That is, given the observed catches (0, 1, 3, 106, 309, 5496), we may expect the distribution of possible catches to vary from a very smooth distribution over the range 0 to 5496 or higher, or to the very rough form implied by the survey where each catch has a one-sixth probability of occurring in the stratum. Four options that span this range of possible distributions are as follows: (*i*) the distribution of catches ranges continuously from 0 to some maximum above 5496 with a small probability of catches above 309; (*ii*) the distribution of catches represents a mixture of two groups, with the first five catches coming from the first group and the large catch of 5496 coming from the second; overlap in the range of catches from the two groups need not be expected and therefore there could be a zero probability for a range of catches somewhere between 309 and 5496; (*iii*) a more extreme case where the distribution of catches is made up of a number (less than six) of nonoverlapping distributions of catches; (*iv*) the distribution implied by fitting an empirical distribution function to the original data, resulting in each observation having a probability of one sixth assigned to it.

Distributions representing the first three options were fitted to the catches from stratum 56 using nonparametric density estimates. The fourth option was already used in constructing the original bootstrap estimates. For the first two options a Gaussian kernel was used with bandwidth estimated by the unbiased cross-validation estimator (Venables and Ripley 1994, p. 139). The distributions for the first option, estimated using an adaptive bandwidth estimate (Silverman 1986, pp. 101–102) and the second, which was estimated with a global bandwidth, are presented in Fig. 8. The original observations are indicated as X's on the abscissa. The distribution obtained with the global bandwidth assigned near-zero probabilities to all observations between 2100 and 4000 fish. The distribution for the third case (Fig. 9) was estimated with the discrete kernel estimate (Rajagopalan and Lall 1995) approach used for the Georges Bank data in the previous subsection. The large spread and sparseness of the observations resulted in four separate groups of discrete distributions when the discrete kernel estimate was used. When ordered from the original data, discrete kernel, global, and adaptive fit, these distributions represent increasing smoothness of the expected distribution of the numbers of haddock caught.

If these distributions are treated as predicting possible catches in the finite population sense, then the impact of these different hypothesized distributions on bootstrap confidence intervals can be evaluated in the following way. Using an approach similar to the smoothed bootstrap (Silverman 1986, p. 143), observations are generated randomly from the fitted distributions for the bootstrap resampling. To keep the observations positive, observations were generated directly from the cumulative density (mass) functions for the distributions in Figs. 8 and 9. A total of 1000 bootstrap replications were made for each of the three distributions for stratum 56 and the BWR method was used for the catches in the other strata.

Quantile–quantile plots of the 1000 bootstrap replications for each distribution are compared with that from using the BWR method (option 4) on all strata in Fig. 10. All of the cases have heavier tails than expected for a normal distribution. The distribution of replications from the distribution using the adaptive bandwidth was the smoothest of the four as expected. The other distributions became progressively rougher as the number of groups increased from two (global bandwidth) through to the original BWR estimates.

The bootstrap estimate of the variance decreased with

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Fig. 8. Probability density functions estimated for the observed catches of haddock (indicated as X's on abscissa) from stratum 56 of the eastern Scotian Shelf survey (1988). Probability density functions were estimated using nonparametric density estimates with Gaussian kernels and unbiased cross-validation bandwidth estimates.

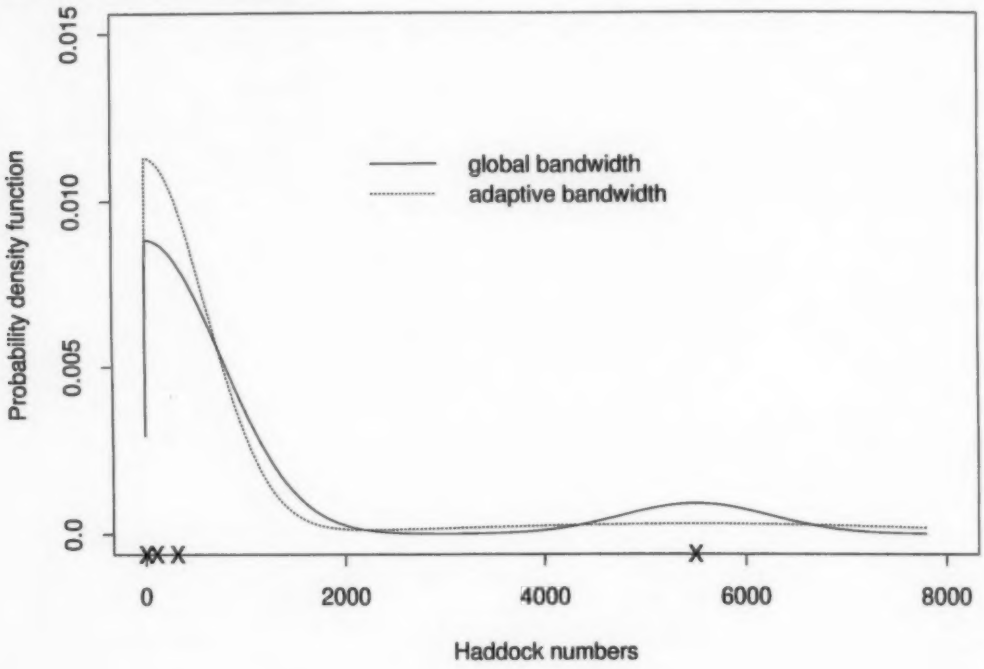

Fig. 9. Probability mass function estimated for the observed catches of haddock (indicated as X's on abscissa) from stratum 56 of the eastern Scotian Shelf survey (1988). Probability mass function was estimated using a discrete kernel estimate and a discrete bandwidth estimated by a cross-validation estimate.

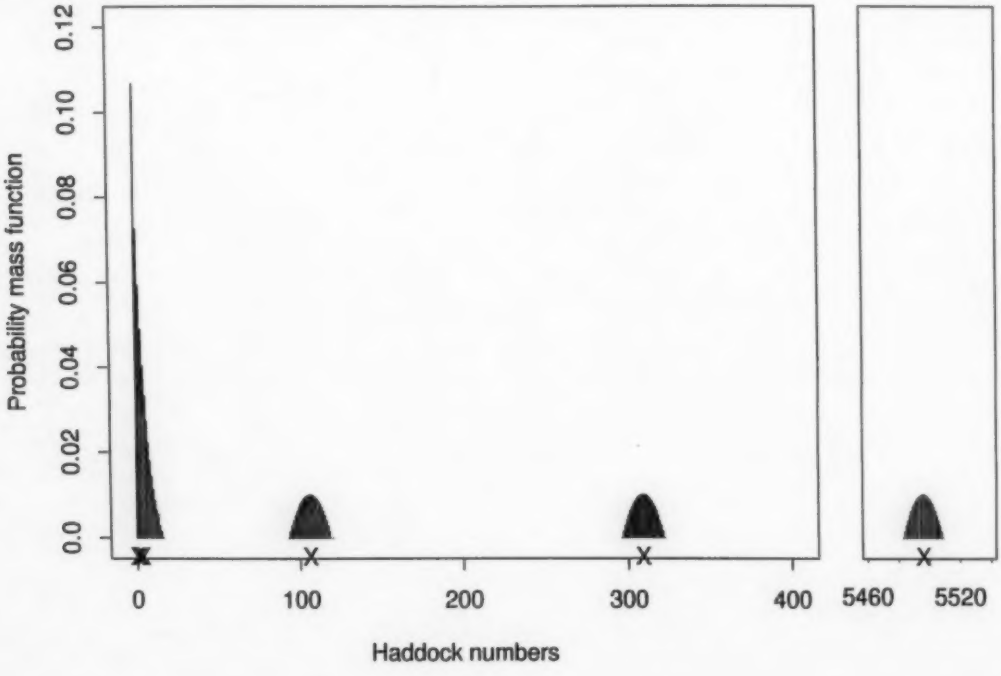

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Fig. 10. Quantile–quantile plots showing the distributions of BWR bootstrap estimates of the stratified mean using all of the catches (standard) and using data simulated from each of the distributions in Figs. 8 and 9.

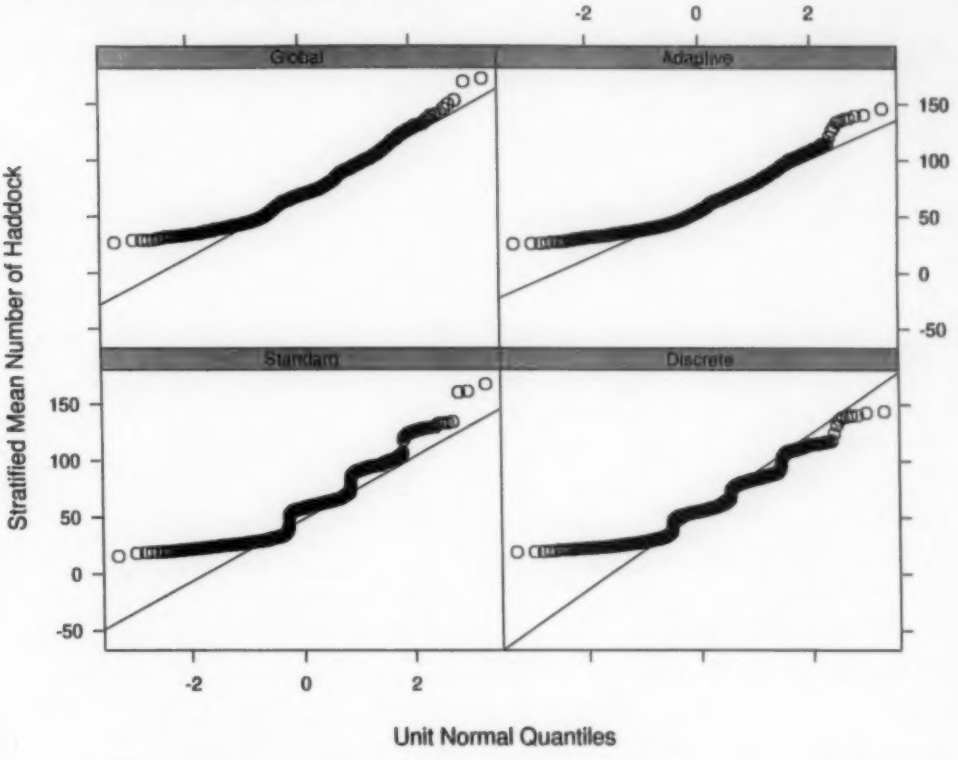

Table 5. Summary of results of the stratified mean, median, variance, and confidence intervals for the number of haddock caught in the 1988 eastern Scotian Shelf survey when nonparametric density estimates are used for the distribution of haddock catch in stratum 56.

				95% confidence interval				
Method	Mean	Median	Variance	Type	Lower	Upper	Length	Shape
Adaptive	58.68	54.22	447.1	PC	31.58	106.74	75.15	0.84
				BC	32.91	114.45	81.55	0.49
Global	69.84	68.33	594.2	PC	34.22	126.21	92.39	0.54
				BC	35.09	131.07	95.99	0.51
Discrete	58.07	55.66	667.3	PC	22.95	114.30	91.35	0.58
				BC	24.32	116.99	92.67	0.43

Note: Three methods are compared: the global bandwidth estimate and adaptive bandwidth estimate (both assuming a continuous distribution) and a Kernel estimate (assuming a discrete distribution). Confidence intervals for the bootstrap mean were calculated using the percentile (PC) and bias-corrected (BC) methods. The length column refers to the length of the confidence intervals. The shape column refers to a measure of symmetry (symmetric: shape = 0) given by Efron (1992).

increasing smoothness of the data (Table 5). The mean was the largest for the global option because of larger density associated with the wide range of the larger catches. The general trend for the PC limits was for narrower limits as the degree of smoothness increased. The upper limit for the PC method from the adaptive distribution was the lowest, but allowing for observations between 309 and 5496 fish (and above) increased the lower limit substantially. The lower limit for the global distribution was also higher than that from the original data but the upper limit was the closest to that from the original data (Table 3). Overall, the effect of assuming increasingly smoother distributions for haddock catches in stratum 56 on the resultant bootstrap PC intervals was to decrease the length and increase the lower limit. Also, comparing the upper PC limit from the adaptive distribution with those from the global and original data suggests that allowing for a continuum of catches over the range observed results in a much lower upper limit. The fitted bandwidth for the discrete distribution was quite narrow and the maximum catch was much lower than those for the global and adaptive distributions. Therefore, the upper PC limit was also lower than those from both the global distribution and the original data.

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The behaviour of the BC limits[1] can be best explained by noting the changing relation between the mean and median for each distribution. For all of the smoothed bootstrap cases the median was less than the bootstrap estimate and hence upper and lower BC limits were greater than their respective PC limits. The reverse was true for the original BWR BC limits (Table 3).

Discussion

The results presented here illustrate that careful attention must be paid to incorporating the sampling scheme into the bootstrap method when dealing with complex designs. The naïve method would appear to be a straightforward extension of the bootstrap from the standard case to sampling with equal probability within each stratum. However, closer inspection shows that this method does not capture all of the aspects of the sampling scheme and an inconsistent estimate of the variance results. Getting the variance of the bootstrap estimates of the mean right is important because these estimates are used to derive the confidence limits.

The studies by Kimura and Balsinger (1985) and Sigler and Fujioka (1988) applied the bootstrap to estimate the abundance of sablefish (*Anoplopoma fimbria*) from complex survey designs that include strata defined by depth. The first study consisted of a pot survey conducted along the west coast of the United States and southeast coast of Alaska whereas the second was a longline survey in the Gulf of Alaska. In the first case, depth strata were referred to but not used in the calculation of abundance and therefore the bootstrap estimates were made assuming all observations had an equal chance of being chosen. However, in the longline survey, the area associated with the depth strata was used in estimating relative population numbers and the application of the bootstrap in this case was similar to the naïve bootstrap. The aim of the bootstrap analysis of the longline data was to detect changes in the estimated relative population numbers from year to year. Given that the naïve bootstrap underestimates the variance, it is probable that more annual differences in abundance were found for the longline survey than actually existed.

Fletcher and Webster (1996) produced results suggesting that a studentized version of the bootstrap augmented with an adjustment for skew will produce more accurate confidence intervals for the stratified mean than the bootstrap alone or the Student t method used here. However, it is difficult to compare their results with those given here because they used the naïve bootstrap and hence underestimated the variance.

The rescale and BWR (mirror-match) methods were designed to capture the second-order term of the Edgeworth expansions of the stratified mean with the bootstrap approximation (Chen and Sitter 1993) and therefore incorporate properties of the original estimates and the sampling scheme. The BWR method appears to be preferred to the rescale method from our results on the basis of simplicity and only being limited to a minimum sample size of $n_h = 2$. Sitter (1992*b*) compared the performance of the rescale methods (for $m_h = n_h - 1$ and $n_h - 3$) with various forms of the mirror-match methods (including BWR) for estimating confidence intervals for functions of stratified means (ratios, regression estimators, and correlation coefficients) and the stratified median. The mirror-match methods performed better on average than the rescale methods with respect to the accuracy of the respective confidence intervals being estimated. The results in Table 4 indicate that PC confidence limits from the BWR method were closest to those expected for the population of stratified means for the Georges Bank data.

All of the bootstrap methods gave insight into the suitability of the Student t distribution for constructing confidence intervals for the stratified mean. The fewer strata, larger sample sizes, and lower variability of the Georges Bank data resulted in the bootstrap limits that appeared to be similar to those given by the Student t distribution. However, the simulation results indicated that the small differences in the bootstrap limits were actually improvements over the Student t limits with respect to tail probabilities and coverage. In addition, these results also argued for the PC confidence interval method being quite adequate for constructing bootstrap confidence intervals for the BWR and rescale ($m_h = n_h - 3$) bootstrap methods.

For the eastern Scotian Shelf data, the relatively large number of strata, small sample sizes within strata, and higher variability resulted in very wide confidence intervals with a negative lower bound for the Student t method. In this case the bootstrap methods offered alternative intervals that were not as wide and resulted in positive lower limits. It was not possible to evaluate the performance of these confidence intervals given the small sample sizes within strata. While the BC_a method corrected the limits in the right direction for a skewed distribution, the simulation results in Table 4 suggest that this correction may be too severe.

All confidence interval methods were affected by the very large catch of haddock in stratum 56 of the Scotian Shelf survey. In practice, there seem to be four ways of dealing with large observations judged to be extreme: keep them, remove them, replace them, or reweight them. Keeping the large observations results in the current situation where we have a large variance, with one or a few catches driving the mean and confidence intervals so large as to be useless. Removing the observation outright is arbitrary and is probably not going to be very popular with the fishing industry: they may consider the one large catch as the only useful catch of the survey. Replacing the large catch(es) with the next largest catch (Smith 1981) or a function of the remaining observations (Moyer and Geissler 1991) could result in a smaller mean-square error but unknown bias.

Reweighting the sets in stratum 56 requires a basis for changing the current weighting of $1/n_h$. As noted before, the catch of 5496 was the largest catch of haddock in the entire time series of the survey to the present day. At 19 m this was the shallowest set made in stratum 56 and the near-bottom temperature of 10.98°C was the highest observed in the whole survey in 1988. In fact, sets have been made at 19 m in stratum 56 only twice in the history of the survey with the second such set made in 1990 resulting in a catch of 3271 haddock at a temperature of 7.36°C. Studies have shown that haddock appear to have strong positive associations with bottom temperature and depth when caught by trawl (Smith et al. 1994). It may be possible to determine the appropriate area weighting for each set on the basis of the expected distribution of depth and

[1] It was not obvious how to calculate the acceleration when smoothed data were used and therefore BC_a intervals were not calculated for these data

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temperature in the stratum. The influence of the large set on the stratified mean and variance would probably be greatly decreased if only a small area of the stratum exhibited these apparently favourable conditions. The bootstrap procedure could be modified for this different weighting of the observations. However, while the depth contours of the stratum could be obtained to determine a depth–catch relationship, temperatures are only known for positions where the sets were made in any one year. A wider sampling of bottom conditions than this would be needed to objectively determine the distribution of temperature (or any other important hydrographic variable) in the stratum.

Are there other options for reweighting the set? When sampling fractions are small and extreme observations are few, Hidiroglou and Srinath (1981) suggest using the following weighting for t extreme observations,

$$\bar{y}'_h = \frac{1}{N_h}\sum_{i=1}^{t} y_{hi} + \frac{N_h - t}{N_h(n_h - t)}\sum_{i=t+1}^{n_h} y_{hi}$$

based on comparing the ratio of its mean-squared to that of the standard estimator of the mean. Without any further guidance, assigning a weight of $1/N_h$ (= 1 / 80 928 for stratum 56) to the large catch may seem extreme and as arbitrary as simply removing the observation. Fitting a statistical distribution such as the Δ distribution (Pennington 1983) is another way of reweighting all of the observations in the stratum on the basis of an assumed mean–variance relationship. The nonparametric density (mass) estimates were used in the same spirit here but offered more flexibility with respect to the shape of the distribution. While six observations are really not enough to characterize a distribution very well, the resultant curves did allow for testing the effects of differently shaped distributions on the bootstrap confidence interval estimates. Use of nonparametric density estimation with bootstrap methods offers a very powerful combination of tools for investigating statistical issues where theoretical support is not well developed.

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Appendix

Stratified estimate of acceleration

Efron and Tibshirani (1993) define the acceleration a as the rate of change of the standard error on the normalized scale. For one-parameter models, they offer the following as a good approximation for a:

$$\hat{a} = \frac{1}{6}\text{skew}_{\theta=\hat{\theta}}(l_\theta),$$

where (l_θ) is the score function. An estimate for this quantity is (Efron and Tibshirani 1993, p. 186)

$$\hat{a} = \frac{\sum_{i=1}^{n}(\hat{\theta}_{(\cdot)} - \hat{\theta}_{(i)})^3}{6\left(\sum_{i=1}^{n}(\hat{\theta}_{(\cdot)} - \hat{\theta}_{(I)})^2\right)^{3/2}}$$

The $\hat{\theta}_i$ refer to the jackknife values of the statistic $\hat{\theta}$ and $\hat{\theta}_{(\cdot)} = \Sigma_{i=1}^{n}\hat{\theta}_i/n$. Note that the denominator and numerator are similar to the second (with power 3/2) and third central moments of $\hat{\theta}$ in the standard definition of skew — μ_3/σ^3. The second central moment for the stratified mean is the variance given in eq. 2, while the third central moment is defined as (Rao and Wu 1988, p. 326)

$$\hat{\mu}_3(\bar{y}_{st}) = \sum_{h=1}^{L} W_h^3(1-f_h)(1-2f_h)\hat{\mu}_{3h}$$

where

$$\hat{\mu}_{3h} = \sum_{i=1}^{n_h}\frac{(y_{hi}-\bar{y}_h)^3}{n_h(n_h-1)(n_h-2)}$$

Replacing s_h^2/n_h and $\hat{\mu}_{3h}$ with the above jackknife quantities for each stratum gives the following as an estimate of the acceleration for a stratified random design:

$$\hat{a}_{st} = \frac{\sum_{h=1}^{L} W_h^3(1-f_h)(1-2f_h)\sum_{i=1}^{n_h}(\hat{\theta}_{h(\cdot)} - \hat{\theta}_{h(i)})^3}{6\left(\sum_{h=1}^{L} N_h(N_h-n_h)\sum_{h=1}^{n_h}(\hat{\theta}_{h(\cdot)} - \hat{\theta}_{h(i)})^2/N^2\right)^{3/2}}$$

In this paper, jackknife values for the stratum means are substituted for $\hat{\theta}_{h(i)}$.

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Scale analyses to estimate somatic growth in sockeye salmon, *Oncorhynchus nerka*

Masa-aki Fukuwaka and Masahide Kaeriyama

Abstract: The relationships between individual growth and scale pattern were examined for juvenile sockeye salmon (*Oncorhynchus nerka*) marked with passive integrated transponder (PIT) tags to assess the usefulness of scale analyses for estimating somatic growth. The relationship between absolute somatic growth and increment of scale radius was linear. The relationship between increment of scale radius and number of circuli was also linear. Path analysis showed that the number of circuli was directly correlated with absolute growth. A negative path coefficient (–0.200) between absolute growth and number of circuli indicated that circulus spacing was positively correlated with somatic growth. The relationship between circulus spacing and absolute growth was linear (circulus spacing (μm) = 0.528 × absolute growth (mm) – 9.57). Results indicate that somatic growth affects circulus spacing directly. Circulus spacing was useful for comparing mean growth from the above equation, while back-calculation was useful for estimating individual growth.

Résumé : Nous avons examiné les relations entre la croissance individuelle et le développement des écailles chez des saumons rouges (*Oncorhynchus nerka*) juvéniles marqués avec des étiquettes à transpondeur passif intégré (PIT) en vue d'évaluer l'utilité de l'analyse des écailles pour estimer la croissance somatique. La relation entre la croissance somatique absolue et l'accroissement du rayon des écailles était linéaire. La relation entre l'accroissement du rayon des écailles et le nombre d'anneaux était aussi linéaire. L'analyse causale a montré que le nombre d'anneaux était directement correlé à la croissance absolue. Un coefficient de cheminement négatif (–0,200) entre la croissance absolue et le nombre d'anneaux indiquait que l'espacement des anneaux était positivement correlé à la croissance somatique. La relation entre l'espacement des anneaux et la croissance absolue était linéaire (espacement des anneaux (μm) = 0,528 × croissance absolue (mm) – 9,57). Les résultats indiquent que la croissance somatique affecte directement l'espacement des anneaux. L'espacement des anneaux a été utile pour comparer la croissance moyenne à partir de l'équation ci-dessus, tandis que le calcul rétrospectif a été utile pour estimer la croissance individuelle.
[Traduit par la Rédaction]

Introduction

Scales are used to estimate age and somatic growth of fish. Circulus spacing is positively correlated with somatic growth in several fish species (Kobayashi 1961; Bilton 1975; Doyle et al. 1987; Fisher and Pearcy 1990). A linear relationship between circulus spacing and somatic growth rate was useful in estimating individual growth rate in an experimental population of tilapia (*Oreochromis mossambicus* and *Oreochromis hornotum*; Doyle at al. 1987), but in coho salmon (*Oncorhynchus kisutch*) it was useful only for comparing somatic growth rate between groups of juveniles (Fisher and Pearcy 1990).

Circulus spacing is determined by both the formation rate of circuli and growth of the scale radius. Bilton (1975) cited Clutter and Whitsel's hypothesis that growth of the scale radius is directly correlated with somatic growth but that circulus formation does not necessarily change at the same rate as somatic growth. However, the effects of somatic growth on circulus formation rate and scale growth are difficult to evaluate. Several authors did not separate the effects of somatic growth on scale growth from the effect of environmental factors (Kobayashi 1961; Bilton and Robins 1971; Ikeda et al. 1974).

Back-calculation is another method of estimating individual somatic growth using scales. Back-calculation is based on proportionality between scale size and body size. However, the relationships between individual scale size and individual body size are variable. Variability of the scale–body size relationship provides bias in back-calculated individual body size. Recent studies minimized such bias using a common original point for proportional back-calculation (Campana 1990; Ricker 1992). Belding (1934) used scale and body sizes at scale formation as the original point in the same fashion as these studies.

To determine whether scale pattern can be used to estimate somatic growth, we examined the relationship between individual growth and scale pattern and compared estimation methods of somatic growth using circulus spacing and back-calculation.

Materials and methods

Sockeye salmon (*Oncorhynchus nerka*) exhibit two life-history patterns: anadromous (sockeye) and nonanadromous (kokanee) types. The anadromous type of sockeye salmon is not endemic to Japan, although the nonanadromous (kokanee) type occurs in several oligotrophic lakes. Anadromous sockeye salmon have been produced from Lake Shikotsu kokanee salmon by smolt release technology in the Bibi River of the Abira River System, central Hokkaido (Kaeriyama 1989). To compare mean somatic growths, we used the two life-history patterns of sockeye salmon.

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Table 1. Means, SDs (in parentheses), variance (diagonal), and correlation (above diagonal) and covariance (below diagonal) matrix of measurement variables used the analysis of kokanee, sockeye salmon, and their hybrids for kokanee egg – kokanee sperm (K–K), kokanee egg – sockeye sperm (K–S), sockeye egg – kokanee sperm (S–K), and sockeye egg – sockeye sperm (S–S) groups.

						Correlation, covariance, and variance						
	K–K	K–S	S–K	S–S	Mean	FL tagging	FL end	SR end	SR check	NC	NC check	Circulus spacing
Fork length (FL) at tagging (mm)	82.0 (6.0)	85.4 (7.1)	76.7 (5.5)	79.5 (6.7)	80.9 (7.0)	49.6	0.805	0.525	0.695	0.384	0.544	0.228
FL at the end of the experiment (mm)	153.1 (7.8)	157.5 (8.0)	146.6 (9.7)	150.5 (10.3)	152 (9.7)	54.6	93.9	0.518	0.525	0.294	0.383	0.379
Scale radius (SR) at the end of the experiment (μm)	813 (104)	830 (85)	737 (85)	790 (78)	794 (94)	346	0.518	8890	0.695	0.641	0.372	0.470
SR at check formation (μm)	341 (60)	366 (59)	279 (61)	312 (56)	325 (66)	322	334	0.695	4390	0.491	0.839	0.361
No. of circuli (NC)	27.5 (2.8)	27.6 (2.8)	26.5 (2.6)	27.8 (1.9)	27.4 (2.6)	6.92	7.29	155	0.491	6.64	0.523	–0.242
NC from the focus to the check	11.9 (1.9)	12.8 (2.4)	10.3 (2.4)	11.4 (2.1)	11.6 (2.3)	8.88	8.60	81.3	129	3.12	5.43	0.145
Circulus spacing during the experiment (μm)	28.4 (2.9)	29.5 (3.0)	26.3 (2.9)	27.4 (2.8)	27.9 (3.08)	4.89	11.2	135	72.7	–1.89	1.03	9.36
No. of individuals	39	37	35	41	152	152	152	152	152	152	152	152

In the fall of 1991, eggs and sperm were stripped from anadromous sockeye salmon returning to the Bibi River and from nonanadromous kokanee in Lake Shikotsu. Four groups of eggs fertilized by the dry method (sockeye egg × sockeye sperm, sockeye egg × kokanee sperm, kokanee egg × sockeye sperm, kokanee egg × kokanee sperm) were incubated in about 8°C water. After emergence, the four groups of juveniles were reared in separate tanks and fed dry pellets. For measurement of individual growth, passive integrated transponder (PIT) tags were injected into the peritoneal cavities of 152 juveniles after measurement of fork length on October 8, 1992 (Table 1). These juveniles were transported to Chitose Hatchery and reared in a circular tank for 214 days under the natural photoperiod.

On May 10, 1993, juveniles were measured (fork length in millimetres), and their scales were collected from the body area near the lateral line between the dorsal and anal fins. Collected scales were mounted on a glass slide in glycerin–gelatin. Scale radius and circulus spacing were measured in micrometres along the longest axis of scale (Fig. 1). A check (decrease in circulus spacing) caused by the stress of the tagging procedure was found by calculating the moving average of circulus spacing (Fukuwaka and Kaeriyama 1994). Scale radius at check formation was equal to the sum of the circulus spacing from the center of the focus to the check.

For the relationship between fork length and scale characters since scale formation, we observed early growth of scale in 43 juvenile kokanee salmon collected at Lake Shikotsu Hatchery on May 17, May 26, and June 24, 1994. To validate the timing of check formation, we plotted the relationships between fork length at tagging and scale characters from focus to the check with the relationships between fork length and scale characters.

The somatic growth rate of juveniles was measured as absolute growth during the experiment (Ricker 1979). Growth of scale radius during the experiment was measured as the increment of scale radius (the difference in scale radius from the check to the outer edge) (Fig. 1). The number of circuli formed during the experimental period was equal to the difference between the total number of circuli and the number of circuli from the focus to the check.

Analysis of covariance (ANCOVA) was used to compare relationships between scale characters and fork length among kokanee, sockeye salmon, and their hybrids. Multiple regression and path analyses were used to examine relationships among absolute growth, increment of scale radius, and the number of circuli formed during the experiment. In path analysis, the effects of predictor variables on a criterion variable are expressed by its path coefficients (Sokal and Rohlf 1981). These coefficients are useful as a relative measure for comparing the magnitude of the effects that different predictor variables have on a criterion variable. We used a t test for the significance of each path coefficient. The results from path analysis are customarily presented in a path diagram, where the effects of predictor variables are depicted by single-headed arrows pointing to the criterion variables.

Geometric mean regression was used to estimate individual growth from circulus spacing. Geometric mean regression of Y on X is exactly the same line as it of X on Y (Ricker 1973). To estimate individual growth, we used the biological intercept back-calculation (Belding 1934; Campana 1990; Ricker 1992): $(S_E - S_0)/(L_E - L_0) = (S_T - S_0)/(L_T - L_0)$, where S_E, S_0, and S_T were scale radii at the end of the experiment, at squamation, and at tagging, respectively, and L_E, L_0, and L_T were fork lengths at the end of the experiment, at squamation, and at tagging, respectively. For S_0 and L_0, we measured scale radius and fork length with no circuli in the scale for five kokanee salmon juveniles (age 0.0) collected at Lake Shikotsu Hatchery in the spring of 1994.

The coefficient of determination of the relationship between observed growth and estimated growth was used to compare these two methods for estimation of individual growth.

Results

Means and standard deviations of measurement variables are indicated in Table 1. The timing of the check formation was validated at tagging by a common relationship consisting visibly of the relationship between fork length and scale radius of

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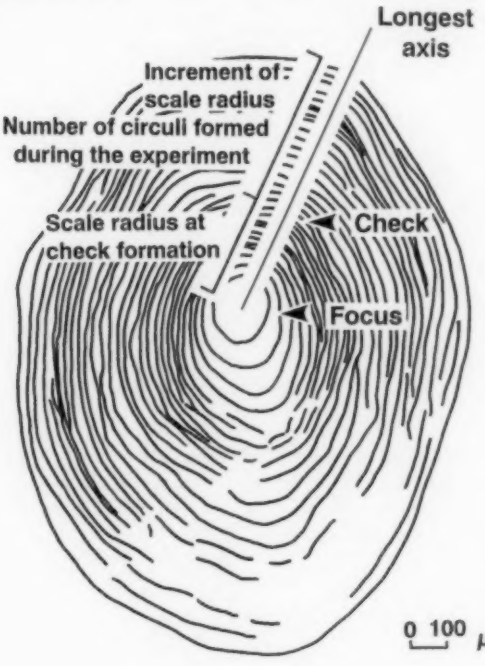
Fig. 1. Scale pattern of sockeye salmon indicating variables used in statistical analyses.



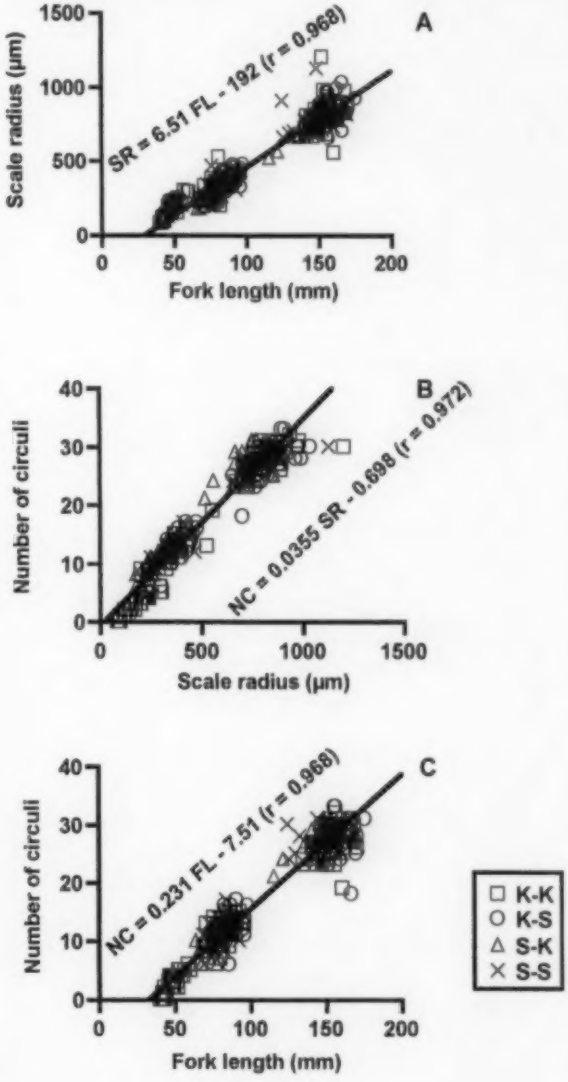
the check (the plotting of the midpoint of Fig. 2A) and the relationship between fork length and scale radius (the plotting of the small and large fishes in Fig. 2A). Relationships between fork length and scale radius and between fork length and number of circuli were linear (Fig. 2). These relationships were not significantly different among kokanee, sockeye salmon, and their hybrids (ANCOVA, $P > 0.05$).

Increment of scale radius was positively correlated with absolute growth during the experiment (Fig. 3A). Number of circuli formed during the experiment was positively correlated with increment of scale radius, but not with absolute growth (Fig. 3). However, number of circuli was a function of both increment of scale radius and absolute growth from multiple regression analysis (ANOVA, $P < 0.001$).

The path analysis showed direct relationships between absolute growth and increment of scale radius, between increment of scale radius and number of circuli, and between absolute growth and number of circuli from path analysis (Fig. 4). The direct effect of absolute growth on number of circuli indicates that absolute growth also affected the relationship between increment of scale radius and number of circuli.

The geometric mean regression was circulus spacing (μm) = 0.528 × absolute growth (mm) – 9.57 ($r = 0.357$, $n = 152$, $P < 0.001$; Fig. 5). Estimated mean growths from circulus spacing were consistent with observed growths in rank order for kokanee, sockeye salmon, and their hybrids (Table 2). Mean fork length of fish that had not yet formed the first scale circulus was 41.2 ± 1.42 mm (mean ± SD; $n = 5$) and mean scale radius was 85.8 ± 6.37 μm ($n = 5$) for Lake Shikotsu kokanee salmon. Back-calculated mean growths did not reflect observed mean growths in rank order (Table 2). However,

Fig. 2. Relationships between fork length, scale radius, and number of circuli of sockeye salmon: (A) fork length – scale radius relationship, (B) scale radius – number of circuli relationship, and (C) fork length – number of circuli relationship for kokanee egg – kokanee sperm (K–K), kokanee egg – sockeye sperm (K–S), sockeye egg – kokanee sperm (S–K), and sockeye egg – sockeye sperm (S–S) crosses. Lines are the geometric mean regressions calculated from pooled data. $n = 347$ for each regression, which was significant at $P < 0.001$.



back-calculated individual growth accounted for more of the variability in observed absolute growth than growth estimated from circulus spacing (Table 3).

Discussion

Kobayashi (1961) suggested that in chum salmon (*Oncorhynchus keta*), circulus number and spacing were related to somatic

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Fig. 3. Relationships between absolute growth, increment of scale radius, and number of circuli formed during experimental period of sockeye salmon: (A) absolute growth – increment of scale radius relationship, (B) increment of scale radius – number of circuli relationship, and (C) absolute growth – number of circuli relationship for kokanee egg – kokanee sperm (K–K), kokanee egg – sockeye sperm (K–S), sockeye egg – kokanee sperm (S–K), and sockeye egg – sockeye sperm (S–S) crosses. Lines indicate the geometric mean regressions calculated from pooled data ($n = 152$). ***, $P < 0.001$; NS, $P > 0.05$.

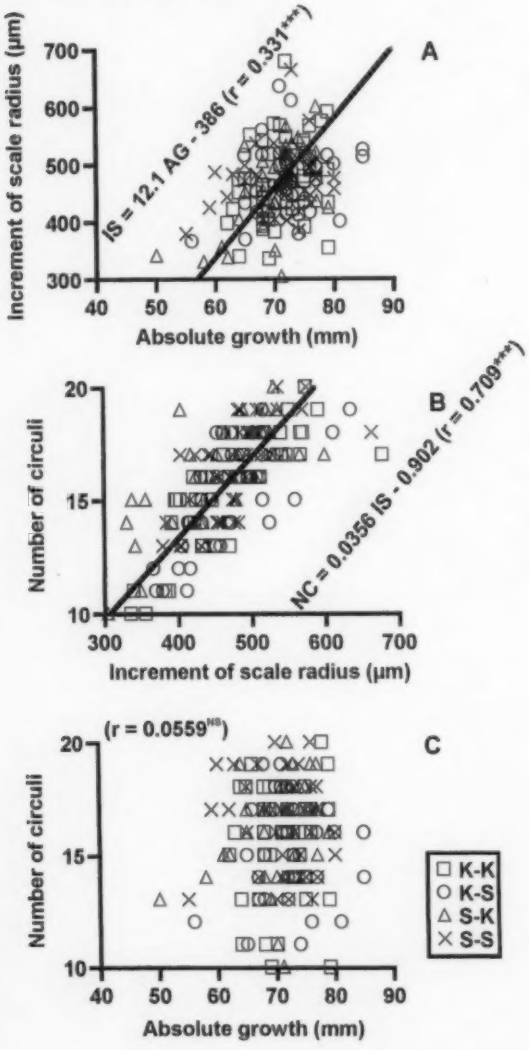


Fig. 4. Path diagram illustrating the relationships among absolute growth, increment of scale radius, and number of circuli for sockeye salmon during the experiment. Path coefficients are printed near the arrows. e_1 and e_2 are residual variables. ***, $P < 0.001$; *, $P < 0.05$.

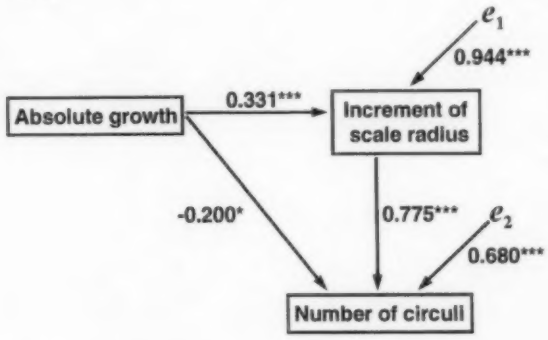
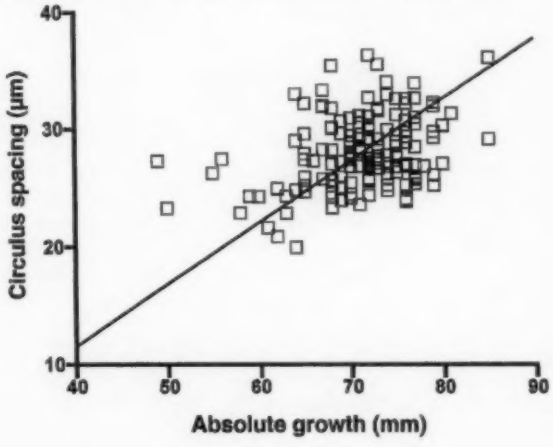


Fig. 5. Relationship between absolute growth and circulus spacing of sockeye salmon during the experimental period. Line indicates the geometric mean regression: circulus spacing (μm) = 0.528 × absolute growth (mm) – 9.57 ($r = 0.357$, $n = 152$, $P < 0.001$).



growth and environmental conditions. Bilton (1975) suggested that circulus spacing was positively correlated with somatic growth in sockeye salmon. Fisher and Pearcy (1990) reported a positive correlation between circulus spacing and somatic growth rate in coho salmon. The present experiment measuring individual growth of juvenile sockeye salmon indicated that circulus spacing was determined by both the rate of circulus formation and growth of scale radius. In addition, somatic growth was directly related to circulus formation. The negative effect of absolute growth on circulus formation indicates a positive relationship between somatic growth and circulus spacing.

The rate of circulus formation is a function of physical and nonphysical factors such as temperature, food, light, genetic factors, and physiological factors (Bilton 1975). Bilton and Robins (1971) reported that circulus spacing increased with feeding level in sockeye salmon. Ikeda et al. (1974) reported that a low-protein diet caused significant narrowing of circulus spacing in goldfish (*Carassius auratus*). Kobayashi (1961) suggested that the difference in circulus spacing between seaward migrants and artificially reared chum salmon was due to different environmental conditions that influence the physiological processes of fish. These studies did not distinguish between the effects of somatic growth and physical environment on circulus spacing. Our results show that somatic growth directly affects circulus spacing. We think that physical

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Table 2. Absolute growth and rank orders estimated and observed during the experimental period in kokanee, sockeye salmon, and their hybrids for kokanee egg – kokanee sperm (K–K), kokanee egg – sockeye sperm (K–S), sockeye egg – kokanee sperm (S–K), and sockeye – egg sockeye sperm (S–S) groups.

	Absolute growth (mm)						
Breeding	Observation	Rank	Circulus spacing	Rank	Back-calculation	Rank	*n*
K–K	71.1±1.5	2	71.9±1.7	2	72.6±2.5	3	39
K–S	72.1±2.0	1	74.0±1.9	1	72.5±2.9	4	37
S–K	69.9±2.0	4	68.0±1.9	4	74.2±3.5	1	35
S–S	71.0±2.1	3	70.0±1.6	3	74.2±2.2	2	41

Note: Values are given as the mean ± 95% confidence limits.

environment affects somatic growth directly and it would affect circulus spacing indirectly. However, we have not evaluated the influence of physical environment on scale pattern in this experiment. Physical environment might affect circulus spacing instead of residual variables in the path diagram (Fig. 4). Another growth experiment is needed to evaluate the influence of physical environment on scale pattern.

We compared two methods estimating growth rates among groups of sockeye and kokanee salmon and their hybrids. Mean growth estimated from circulus spacing reflected actual mean growth. Back-calculated individual growth was more representative of actual individual growth than growth estimated from circulus spacing (Table 3). Fisher and Pearcy (1990) suggested that circulus spacing was useful for comparing growth rates between groups of juvenile coho salmon if the fish were similar in size and age and if the relationship between scale radius and body length was similar. Our results suggested that circulus spacing would provide better estimated mean growth than back-calculation for sockeye salmon because their growths have a narrow range. Circulus spacing would be useful for comparing mean growths of groups of sockeye salmon.

The strong relationship between fork length and scale radius should induce the correlation between somatic growth and scale growth. The correlation coefficient of the relationship between absolute growth and increment of scale radius (0.331) was smaller than that of the relationship between fork length and scale radius (0.968; Fig. 3). The relationship between absolute growth and increment of scale radius was individually variable. Other relationships between somatic growth, scale formation, and circulus formation were also variable among individuals. The weak relationship between absolute growth and circulus spacing ($r = 0.357$) was attributed to the individual variability of the relationship between somatic growth and scale growth (Fig. 4, Table 3). The geometric mean regression was averaged individual variability in the relationship. Estimated individual growth would, therefore, be strongly affected by individual variability in the relationship, whereas estimated mean growth would be less affected. The confidence intervals of estimated growth from circulus spacing were smaller than those of observed growth (Table 2). While the individual variabilities of growth would offset each other in the estimates by circulus spacing, it would be reflected in the estimates by back-calculation.

Scale characteristics provide the information on somatic growth in sockeye salmon. Growth estimation by scale analyses

Table 3. Coefficients of determination between observed growth and estimated growths in *Oncorhynchus nerka*.

Method for growth estimation	Coefficient of determination	No. of individuals	*P*
Back-calculation	0.414	152	<0.001
Circulus spacing	0.127	152	<0.001

may be used not only in the investigation of life history and population dynamics but also in the assessment of the growth environment of sockeye salmon in river, lake, and ocean habitats. We think that circulus spacing should be used for growth estimation and comparisons only when fish growths are in a narrow range. Back-calculation should be used to estimate individual growth. The back-calculation technique uses various marks on the scale such as annual rings or checks caused by the stress of release of hatchery fish (Kobayashi 1961; Fukuwaka and Kaeriyama 1994). Field studies often lack these time markers. Healey (1982) claimed that there was size-selective mortality of juvenile chum salmon by using a comparison of circulus spacing. Circulus spacing may be used for rough comparisons of growth rates in the short period before the time marker (annulus or growth check) has formed.

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Indirect effects of lake acidification on *Chaoborus* population dynamics: the role of food limitation and predation

Janet M. Fischer and Thomas M. Frost

Abstract: A twofold increase in *Chaoborus punctipennis* abundance following experimental acidification of one basin of Little Rock Lake, Wisconsin, provided an opportunity to examine the hypotheses that *C. punctipennis* dynamics were regulated by (*i*) food resources for instars I and II, (*ii*) fish predation on instars III and IV, and (*iii*) invertebrate predation on instars I and II. Neither food limitation nor fish predation appeared to be important constraints on *C. punctipennis* abundance. Although rotifer biomass increased significantly during the acidification, *C. punctipennis* did not respond in a consistent manner to variation in food availability. Bioenergetics estimates of *C. punctipennis* consumption by rock bass (*Ambloplites rupestris*), the most acid-sensitive fish species, were small compared with *C. punctipennis* production. In contrast, invertebrate predation appeared to be an important constraint on *C. punctipennis* abundance. *Chaoborus punctipennis* abundance increased dramatically when abundance of the predator *Mesocyclops edax* declined to near zero. Comparison of *M. edax* predation rates and first instar production suggested that *M. edax* could exert strong predation pressure on *C. punctipennis* Thus, invertebrate predation may create a juvenile bottleneck for populations of small-bodied *Chaoborus* species in moderately productive acidified lakes.

Résumé : Un doublement de l'abondance de *Chaoborus punctipennis* à la suite de l'acidification expérimentale d'un bassin du lac Little Rock (Wisconsin) nous a donné l'occasion d'examiner les hypothèses selon lesquelles la dynamique de *C. punctipennis* serait régulée par (*i*) les ressources alimentaires pour les stades larvaires I et II, (*ii*) la prédation par les poissons sur les stades III et IV et (*iii*) la prédation par les invertébrés sur les stades I et II. La limitation trophique, pas plus que la prédation par les poissons, ne semblait exercer une contrainte importante sur l'abondance de *C. punctipennis* Bien que la biomasse des rotifères se soit accrue de façon marquée pendant l'acidification, *C. punctipennis* n'a pas réagi de façon uniforme à la variation de la disponibilité de la nourriture. Les effets bioénergétiques de la consommation de *C. punctipennis* par le crapet de roche (*Ambloplites rupestris*), poisson le plus sensible à l'acidification, étaient faibles comparés à la production de *C.s punctipennis* Par contre, la prédation par des invertébrés semblait exercer une contrainte importante sur l'abondance de *C. punctipennis* Cette abondance s'est accrue de façon spectaculaire quand l'abondance du prédateur *Mesocyclops edax* est tombée à près de zéro. La comparaison des taux de prédation par *M. edax* et de la production du premier stade permet de penser que *M. edax* peut exercer une forte pression de prédation sur *C. punctipennis* Ainsi, la prédation par les invertébrés peut créer un goulot d'étranglement au niveau des juvéniles pour les populations d'espèces de *Chaoborus* à petit corps dans les lacs acidifiés modérément productifs.
[Traduit par la Rédaction]

Introduction

Acidification has caused dramatic shifts in species composition of aquatic communities throughout North America (Baker and Christensen 1991). For some species, declines in abundance can be attributed to direct effects of pH on survival, reproduction, and (or) physiological function (Swenson et al. 1989; Webster et al. 1992). In many cases, however, changes in population abundance occur as indirect responses to changes in the food web, e.g., changes in prey or predator abundance (Yan et al. 1985; Webster et al. 1992; Locke and Sprules 1993; Gonzalez and Frost 1994). Understanding the mechanisms underlying population responses to acidification can be a major challenge, particularly when population responses occur largely as a result of indirect effects.

The phantom midge, *Chaoborus* spp., often increases in abundance in acidified lakes (Eriksson et al. 1980; Yan et al. 1982, 1985, 1991). Laboratory investigations do not reveal a direct effect of acid on *Chaoborus* survival at pH levels typical of acidified lakes (reviewed in Locke 1991). In addition, a survey of lakes that span a pH gradient suggests that pH is not a prime determinant of *Chaoborus* abundance (Yan et al. 1985). Rather, increases in *Chaoborus* abundance with acidification occur largely as a result of indirect effects of changes in the food web.

Chaoborus has a classic stage-structured life history featuring changes in size and habitat use. As *Chaoborus* develops, larvae pass through four instar stages during which their average body length increases approximately fourfold (LaRow and Marzolf 1970). First and second instars inhabit the lower depths of the epilimnion (Moore 1988). In contrast, third and fourth instar larvae exhibit pronounced diel vertical migration, inhabiting the hypolimnion or the sediments during the day

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and the epilimnion at night (LaRow and Marzolf 1970). The fourth instar larva molts to a pupal stage, which migrates to the water surface and emerges as an adult midge.

Ecological constraints on *Chaoborus* survivorship also shift with ontogeny. Food limitation can be an important constraint on survivorship of first instars. The small mouth gape diameter of early instar larvae (I and II) limits them to feeding primarily on rotifers (usually <200 μm in width) and large flagellated phytoplankton (Moore 1988; Yan et al. 1991). However, later instars (III and IV) can also feed on large rotifers like *Asplanchna* spp., and small crustaceans including *Bosmina*, *Diaphanosoma*, and some *Daphnia* and copepod species (Elser et al. 1987; Moore 1988; Yan et al. 1991). Consequently, the importance of food limitation declines dramatically in later instar stages (Neill and Peacock 1980; Elser et al. 1987; Moore 1988; Neill 1988).

Predation by vertebrate and invertebrate predators can also strongly constrain *Chaoborus* abundance. Fish are size-selective predators and may strongly affect survivorship of later instars (III and IV) (Pope et al. 1973; Northcote et al. 1978; Stenson et al. 1978; Eriksson et al. 1980; Elser et al. 1987). While early instars (I and II) have a size refuge from fish predation, they are consumed by invertebrate predators such as predatory copepods (Sprules 1972; Dodson 1974; Williams 1980; Fischer and Moore 1993). In a particular lake, population dynamics of *Chaoborus* spp. are controlled by the suite of processes that determine survivorship of each life stage.

Processes that affect survivorship of different stages of the complex life history of *Chaoborus* can interact to determine the response of *Chaoborus* species to acidification. While numerous studies have linked changes in *Chaoborus* abundance to complete elimination of acid-intolerant fish predators (Eriksson et al. 1980; Yan et al. 1982, 1985, 1991), responses of *Chaoborus* to reduced fish predation may depend on lake trophic status. In a survey of lakes in central Canada, Yan et al. (1985) documented high *Chaoborus* spp. abundance in nutrient-rich acidified lakes where the combined effects of abundant prey for first instars and elimination of fish predators permit high survivorship of *Chaoborus*. In contrast, *Chaoborus* spp. abundance is often low in nutrient-poor acidified lakes (Yan et al. 1985). Although fish predators have been eliminated, prey resources for early instars are insufficient in these less productive lakes. Yan et al.'s (1985) results demonstrate the importance of evaluating the role of several factors (e.g., predation and food limitation) when investigating the response of *Chaoborus* spp. to acidification.

During the experimental acidification of Little Rock Lake, Wisconsin, to pH 4.7, *Chaoborus* spp. abundance increased approximately twofold (Sierszen and Frost 1993). Intensive monitoring of the food web in the acidified and reference basins provided an opportunity to examine the factors regulating *Chaoborus* spp. abundance in a moderately productive (total nitrogen 90 $\mu g \cdot L^{-1}$, total phosphorus 9.0 $\mu g \cdot L^{-1}$; Brezonik et al. 1986) acidified lake. Because the manipulation was carried out at the scale of a lake basin, experimental artifacts arising from scale-dependent interactions between predators and prey were avoided. We examined the relative importance of three nonexclusive hypotheses for the observed increase in *Chaoborus* spp. abundance in the treatment basin: (*i*) decreased food limitation for early instars, (*ii*) decreased fish predation on later instars, and (*iii*) decreased invertebrate predation on early instars. Our evaluation of the role of these factors in regulating *Chaoborus* spp. abundance was based on analysis of changes in food and predator abundance and an assessment of the potential magnitude of food limitation and predation impacts.

Methods

Study site

Little Rock Lake, an 18-ha lake located in the Northern Highland Lake District of Wisconsin (Vilas County, 45°59′55 N, 89°42′15 W), is the site of an ongoing investigation of the effects of acidification on lake ecosystems. The two basins of Little Rock Lake were separated with an inert plastic curtain in 1984. Beginning in 1985, the pH of the lake's northern basin was reduced from an initial pH of 6.1 in a series of three steps lasting 2 years each. Target values of pH 5.6, 5.1, and 4.7 were attained during the acidification. Experimental acidification was completed in 1990, and the treatment basin is currently in a recovery phase where chemical and biological conditions are returning to preacidification levels. A detailed description of Little Rock Lake's basic limnology and a summary of lake responses to acidification are provided by Brezonik et al. (1993).

Chaoborus abundance and production

The abundance of *Chaoborus* spp. was monitored in treatment and reference basins during acidification and recovery (Sierszen and Frost 1993). In Little Rock Lake, *Chaoborus* species consist almost entirely of *Chaoborus punctipennis* (>95%), with rare occurrences of *Chaoborus albatus*. Hereafter, we will refer to *Chaoborus* spp. in Little Rock Lake as *Chaoborus punctipennis* to reflect the dominance of this species in controlling *Chaoborus* dynamics. Routine sampling was conducted approximately biweekly at a permanent sampling station at the deepest point in the lake roughly 1 h after sunset. Vertical tows were taken from 0.5 m above the lake bottom to the surface of the lake with a plankton net (0.5 m diameter, 1.5 m long, 253-μm mesh). Because of the relatively large mesh size of the plankton net, early instar abundance was probably underestimated. *Chaoborus punctipennis* larval stages were not distinguished when the samples were counted.

To obtain estimates of instar-specific *C. punctipennis* production, abundance of early instar stages was quantified by weekly sampling during 1992 and 1993. We used two net sizes to maximize sampling efficiency for different instar stages. Instars I and II were counted in samples collected with a 148-μm mesh plankton net, while instars III and IV were assessed in 253-μm mesh net samples. Both nets were 0.5 m in diameter and 1.5 m long. All samples were preserved with Lugol's iodine solution and counted using a dissecting microscope at 12×.

Correction factors based on the mean of July 1992, July 1993, and August 1993 net efficiency comparisons with integrated water column samples were applied to 1992 and 1993 raw count data. Net tows and integrated water column samples were collected at night from 0–4 and 0–8 m depth in the reference and treatment basin, respectively. The integrated water column sample was pooled from a series of contiguous Schindler–Patalas trap (2 m long, 90 L, 83-μm mesh) samples. Net efficiencies for instars I through IV in the reference basin were 32, 62, 100, and 92%, respectively. In the treatment basin, net efficiencies for instars I through IV were 32, 65, 72, and 48%. To maintain consistency in the long-term record, abundance of *C. punctipennis* larvae during 1992 and 1993 was also assessed using 253-μm mesh net samples as in previous years.

Chaoborus punctipennis production in 1992 and 1993 was calculated according to the method of Southwood (1978). Production for each larval stage (P_i) was estimated as

$$P_i = (A_i/D_i)\Delta M_i$$

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Table 1. Estimated biomass gain (ΔB), development time (D), growth rate (G), respiration rate (R), assimilation efficiency (AE), and consumption rate (C) for first instar *Chaoborus punctipennis* in Little Rock Lake.

Basin	ΔB (µg)	D (days)	G (µg·day^{-1})	R (cal·day^{-1})	AE* (%)	C (µg·individual^{-1}·day^{-1})
Treatment	6.6	5	1.33	0.001	54	3.44
Reference	7.4	7	1.01	0.001	54	2.68

*Data are from Cressa and Lewis (1986).

where A_i is the area under the abundance versus time curve, D_i is development time, and ΔM_i is change in mass. Development times were estimated using cohort analysis (Braner and Hairston 1989). Instar-specific measurements of mass were not determined for 1993; therefore, we used measurements from 1992 in the 1993 calculation. Because individual masses were lognormally distributed, mass measurements were log-transformed prior to calculation of change in mass (ΔM_i). Change in mass within an instar was calculated as 4 SDs (upper size = mean + 2 SDs; lower size = mean – 2 SDs).

Zooplankton abundance

Zooplankton abundance and taxonomic composition were monitored in treatment and reference basins of Little Rock Lake throughout the experiment. During open water periods, zooplankton were collected biweekly using a 33-L Schindler–Patalas trap (53-µm Nitex mesh) at the permanent sampling stations (Frost and Montz 1988). All samples were collected between 10:00 and 16:00, on the same day in both basins. Samples were collected from the epi-, meta-, and hypo-limnion (0, 4, and 8 m in the treatment basin and 0, 4, and 6 m in the reference basin) and preserved with 4% sucrose-buffered formalin. Hypsometrically weighted average abundances were calculated for each basin. For rotifer taxa, hypsometrically weighted average biomass was calculated using measured lengths in Little Rock Lake and the volume–weight relationships given in Ruttner-Kolisko (1977).

Testing alternative mechanisms for regulation of *Chaoborus* population dynamics

Food limitation

To test the hypothesis that the dramatic increase in *C. punctipennis* abundance in the treatment basin was driven by increased food resources for early instars, we examined trends in edible rotifer biomass from 1985 through 1993. If food resources were an important constraint on *C. punctipennis* dynamics, we expected that prey availability would increase in the treatment basin immediately preceding or coincident with the increase in *C. punctipennis*. Because the mouth gape diameter of first instar *C. punctipennis* is approximately 100 µm (Moore 1988), we assumed that first instars were limited to feeding on rotifers less than 75 µm in width. Colonial taxa and rotifers embedded in a gelatinous matrix were considered to be inedible (Moore 1988). Edible rotifer taxa determined using these criteria included *Ascomorpha* sp., *Conochilus* sp., *Gastropus stylifer*, *Kellicottia bostoniensis*, *Keratella cochlearis*, *Keratella cochlearis* var. *tecta*, *Keratella hiemalis*, *Keratella taurocephala*, *Lecane* sp., *Polyarthra remata*, *Polyarthra vulgaris*, and *Trichocerca birostris*. While previous studies have shown that early instars also consume phytoflagellates, these prey items are generally a small (<1%) proportion of the biomass-based diet in mesotrophic lakes like Little Rock (Moore 1988; Moore et al. 1994).

In addition, we estimated the food requirements of first instar *C. punctipennis* using a bioenergetics approach and compared these estimates to prey availability. If prey availability exceeded the food requirements, we inferred that food resources were not limiting. In 1992 (the only year where estimates of both first instar abundance and growth rates were available), the mean consumption (C) rate of an individual first instar was estimated using a mass balance equation:

$$C = (G + R)/\mathrm{AE}$$

where G, R, and AE represent growth, respiration, and assimilation efficiency, respectively (Elser et al. 1987; Saunders and Lewis 1988; Yan et al. 1991). Total food requirements of first instar *C.punctipennis* in Little Rock Lake were calculated as the product of individual consumption rates (Table 1; see also Fischer 1994) and first instar abundance.

Fish predation

The fish community in Little Rock Lake is dominated by four species: yellow perch (*Perca flavescens*), largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*), and rock bass (*Ambloplites rupestris*) (Swenson et al. 1989). The sequence of changes in the fish community of Little Rock Lake suggested that a change in rock bass abundance may have been responsible for the increase in *C. punctipennis* abundance in the treatment basin (Swenson et al. 1989; Eaton et al. 1992; W.A. Swenson, University of Wisconsin-Superior, Superior, Wis., unpublished data). Yellow perch, the dominant planktivore in the lake, was insensitive to the acidification and exhibited only a slight reduction in juvenile recruitment at pH 4.7 (Eaton et al. 1992; S. Newman, Wisconsin Department of Natural Resources, Madison, Wis., unpublished data). In addition, *Chaoborus* spp. constituted a small proportion of yellow perch diets (<5% by number consumed) and the number of *C. punctipennis* in the diets of yellow perch from the treatment basin did not differ significantly among years during the period 1985–1988 (analysis of variance (ANOVA), df = 3, $F = 2.05$, $p > 0.1$; W.A. Swenson, unpublished data; J.M. Fischer, unpublished data). Largemouth bass recruitment was reduced at pH 5.1; however, significant recruitment failure was observed only at pH 4.7 (Eaton et al. 1992; S. Newman, unpublished data). Furthermore, *C. punctipennis* was a small component (<0.2%) of young-of-the-year and age-1 largemouth bass diets (S. Newman, unpublished data). Black crappie were unlikely to strongly influence *C. punctipennis* dynamics because they were rare in Little Rock Lake (<90 adult fish/basin) (Eaton et al. 1992).

In contrast to the other fish species, rock bass responded sensitively to acidification, exhibiting reduced recruitment at the first step of the acidification and complete recruitment failure thereafter. *Chaoborus punctipennis* can constitute up to 50% (by number consumed) of the diets of age-2+ and older rock bass (W.A. Swenson, unpublished data). It is important to note, however, that rock bass make up a small proportion of the fish community in Little Rock Lake (<600 fish/lake basin). We did not expect changes in rock bass predation to have a major impact on *C. punctipennis* population dynamics but included the analysis for the sake of completeness. If fish predation was an important constraint on *C. punctipennis* dynamics, we expected that (*i*) rock bass consumption would decrease in the treatment basin immediately preceding or coincident with the increase in *C. punctipennis*, and (*ii*) the magnitude of *C. punctipennis* consumption by rock bass during 1985–1987 would be similar to or exceed estimates of production for later instars.

We used the bioenergetics approach of Kitchell et al. (1977) to estimate rock bass consumption of *C. punctipennis* during 5 years (1985–1989), which bracketed the increase in *C. punctipennis* abundance in the treatment basin in 1988. These years were chosen because they were the only years in which rock bass abundance and growth rates were monitored in Little Rock Lake. We estimated *Chaoborus* consumption by rock bass from rock bass abundance, growth rates, diet, and water temperature in Little Rock Lake using the generalized bioenergetics software of Hewett and Johnson (1992),

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Table 2. Mean weight at age (g) for rock bass in Little Rock Lake during 1983–1990.

	Age							
Basin	1	2	3	4	5	6	7	8
Reference	1.62±0.51	12.14±2.23	42.08±5.59	67.93±14.45	89.21±8.36	97.39±7.38	125.97±3.19	121.94±0.49
Treatment	1.52±0.48	11.71±2.21	40.90±2.75	69.69±8.27	89.83±5.00	102.94±11.19	128.61±14.19	150.42±2.25

Note: Values are given as the mean ± SD. Year-specific values were used in bioenergetics simulations.

with rock bass parameters from Roell and Orth (1993). During 1985–1989, abundance of rock bass age 2+ and older was estimated using mark–recapture techniques. Abundance of age-1+ fish was hindcast from the abundance of age-2+ fish using an annual mortality rate of 50%. This annual mortality rate was estimated by fitting an exponentially declining curve to abundance versus age data. Size at age was determined by back-calculation from scale annuli. When scales were not available for an age-class, we assumed a mean size on the basis of estimates from all years in which back-calculated sizes were available. Size at age was converted to biomass using the following length–weight regression for Little Rock Lake rock bass (W.A. Swenson, unpublished data):

$$\log_{10} W = 2.92 \log_{10} L - 4.60, \quad n = 90, r^2 = 0.97$$

where W is weight in grams and L is total length in millimetres (see Table 2 for average weight at age in Little Rock Lake). We deliberately overestimated rock bass consumption of *C. punctipennis* by assuming that (*i*) all age-classes of rock bass feed exclusively on *C. punctipennis*; (*ii*) all growth occurred between 20 May and 7 October, the period when epilimnetic temperatures were >10°C; and (*iii*) no mortality occurred during the summer growing season. While rock bass were not intensively sampled after 1989, a fish survey conducted in 1995 suggests that rock bass have not recovered in the treatment basin (J.M. Fischer, unpublished data).

To assess the potential impact of rock bass predation on *C. punctipennis*, bioenergetics estimates of consumption were compared with production estimates for later instar *C. punctipennis*. *Chaoborus punctipennis* production was estimated using data from 1992 and 1993, the only years in which instar-specific densities and growth rates were measured (see *Chaoborus* abundance and production section). We believe that estimates from 1992 and 1993 provide a rough indication of *C. punctipennis* production in previous years.

Invertebrate predation

To evaluate the potential for invertebrate predation to drive *C. punctipennis* dynamics in the treatment basin, we examined changes in the abundance of the copepod *Mesocyclops edax* from 1985 through 1993. We focused on *M. edax* because adults of this species are voracious predators capable of consuming a wide range of zooplankton (Williamson and Magnien 1982), including first instar *C. punctipennis* (Fischer and Moore 1993). *Mesocyclops edax* is also the most abundant invertebrate predator in Little Rock Lake and overlaps temporally and spatially with first instar *C. punctipennis*. The invertebrate predators *Epischura lacustris* and *Leptodora kindtii* also occur in Little Rock Lake. However, the low abundance of *E. lacustris* and *L. kindtii*, as well as the timing of their response to acidification, makes it unlikely that these species were responsible for the *Chaoborus* increase (Sierszen and Frost 1993). If invertebrate predation by *M. edax* was an important constraint on *C. punctipennis* dynamics, we expected that (*i*) the density of *M. edax* would decrease in the treatment basin preceding or coincident with the increase in *C. punctipennis* and (*ii*) estimates of the predation by *M. edax* on first instar *C. punctipennis* would be similar to or exceed first instar production.

We estimated the magnitude of predation by *M. edax* on first instar *C. punctipennis* in Little Rock Lake using the method of Williamson and Stoeckel (1990). Predation rate estimates are based on predator density, laboratory-based predation rates, and an index of overlap of predator and prey. In our calculations, we used predation rates from the laboratory experiment of Fischer and Moore (1993). In this experiment, predation by adult female *M. edax* on first instar *C. punctipennis* was assessed in the presence of alternative prey. Predation rates ranged from zero to two first instar *C. punctipennis* per *M. edax* each day (two first instar *C. punctipennis* per *M. edax* each day was the maximum predation rate possible given the experimental design). Density of first instar *C. punctipennis* in the laboratory experiment ($2 \cdot L^{-1}$) was higher than typical densities in Little Rock Lake ($<0.1 \cdot L^{-1}$). However, predation experiments conducted using lower *C. punctipennis* densities ($0.25 \cdot L^{-1}$) yield similar predation rates (J.M. Fischer, unpublished data). In our calculations, we assumed an average predation rate of 0.5 first instar *C. punctipennis* per *M. edax* each day. Spatial overlap (O) was calculated from vertical distributions of *M. edax* adults and first instar *C. punctipennis* during June and July 1993 using the approach of Williamon and Stoeckel (1990):

$$O = \frac{\sum_{z=1}^{m} (N_{jz}\, n_{iz})m}{\sum_{z=1}^{m} (N_{jz}) \sum_{z=1}^{m} (n_{iz})}$$

where N_j is the density of predator species j, n_i is prey density, m is the total number of depths sampled, and z is the individual depth sampled. To evaluate the potential of *M. edax* to control *Chaoborus* dynamics in Little Rock Lake, we contrasted predation estimates and first instar production.

Statistical analyses

Because whole-lake experiments are rarely replicated, statistical analysis of changes following manipulation requires nonconventional statistical techniques (Carpenter et al. 1989). Randomized intervention analysis (RIA; Carpenter et al. 1989; Carpenter 1993) is a useful analytical tool for detecting changes in a manipulated ecosystem relative to a reference ecosystem. We used RIA to examine the probability that the observed changes in *C. punctipennis* abundance, edible rotifer biomass, and *M. edax* abundance were due to chance. We performed a series of planned contrasts designed to test for differences between lake basins occurring between (*i*) the first step and subsequent steps of acidification (1985–1986 vs. 1987–1990) and (*ii*) the first two steps and the final step of the acidification (1985–1988 vs. 1989–1990). Because many of the biological changes in Little Rock Lake occurred during the 2nd year of an acidification step, we also contrasted 1985–1987 with 1988–1990. Although results of RIA can be sensitive to temporal autocorrelation (Steward-Oaten et al. 1992; Carpenter 1993), RIA test results were not altered by autocorrelation for any of the planned comparisons.

Results

Chaoborus abundance

Chaoborus punctipennis abundance increased significantly during the second step of the acidification of the treatment basin (Fig. 1, Table 3). In 1988, the 2nd year of the pH 5.1 phase of the acidification, *C. punctipennis* abundance was approximately two times higher in the treatment basin than in the

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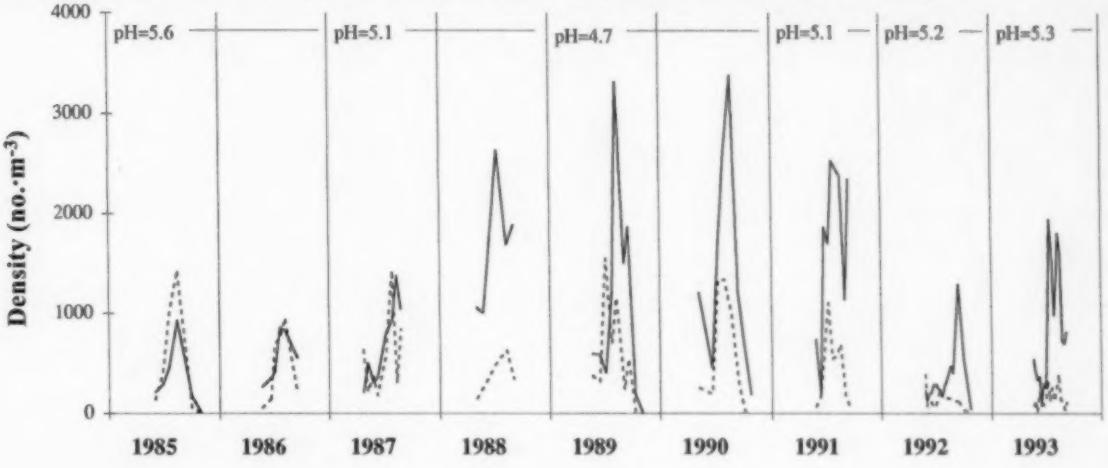
Fig. 1. Abundance of *C. punctipennis* larvae in the reference (broken lines) and treatment (solid lines) basins of Little Rock Lake during the summer-stratified season. *Chaoborus* samples were collected using a 253-μm plankton net, and all larval stages were pooled.



reference basin. In 1991, immediately following the completion of acid additions to the treatment basin, *C. punctipennis* persisted at a high level relative to the reference basin. In 1992 and 1993, differences in *C. punctipennis* abundance between basins, although still apparent, declined substantially.

Food limitation

There was little evidence to suggest that increased food supply was the major factor driving the *C. punctipennis* population increase in Little Rock Lake. Biomass of rotifers in the size range consumed by first instar *C. punctipennis* increased significantly with the acidification of the treatment basin; however, this increase was not observed until 1989, 1 year after the dramatic increase in *C. punctipennis* (Fig. 2, Table 3). It is unlikely that there were substantial changes in rotifer productivity in 1988. Gonzalez et al. (1990) reported that birth rates of the dominant rotifer species in Little Rock Lake (*Keratella cochlearis* and *Keratella taurocephala*) in 1988 were similar to or lower than those in previous years.

The largest between-basin differences in edible rotifer biomass occurred in the early summer, before the time when first instar *C. punctipennis* first appeared in the water column. In Little Rock Lake, first instar *C. punctipennis* appeared following two pulses of emergence and reproduction in mid-June and July (Fischer 1994). During this critical midsummer period, the average biomass of edible rotifers was similar between basins (2.18 ± 1.18 and 2.82 ± 1.52 μg·L^{-1} (mean ± SD) in the reference and treatment basin, respectively). Furthermore, *C. punctipennis* population abundance did not respond in any consistent manner to variation in midsummer food availability in either basin. Specifically, the correlation between average midsummer edible rotifer biomass (weighted by the inverse of the variance to account for differences in number of midsummer sampling dates among years) and average summer *C. punctipennis* abundance was low and insignificant ($r = 0.09$, $p > 0.70$).

Finally, bioenergetics calculations for 1992 suggested that edible rotifer biomass exceeds the food requirements of first instar *C. punctipennis* (Fig. 3). In the reference basin, food availability exceeded the maximum food requirements of *C. punctipennis* by more than 13-fold. Although food requirements of *C. punctipennis* were higher in the treatment basin, food availability still exceeded the requirements of first instar *C. punctipennis*. Thus, neither the record of edible rotifer biomass nor the bioenergetics assessment of the early instar food requirements suggests that food limitation was responsible for the dramatic increase in *C. punctipennis* abundance in Little Rock Lake.

Table 3. Probability values for RIA comparisons of *C. punctipennis* abundance, edible rotifer biomass, and *Mesocyclops edax* abundance.

	1985–1986 vs. 1987–1990	1985–1987 vs. 1988–1990	1985–1988 vs. 1989–1990
Chaoborus spp.	0.004	<0.001	0.079
Rotifers	0.096	0.048	<0.001
M. edax	0.504	0.004	0.003

Note: Values indicate the probability that the change in the difference between treatment and reference basins was random.

Fish predation

Bioenergetics assessment of rock bass predation did not support the hypothesis that changes in *C. punctipennis* abundance were driven by reduced predation on later instars. Rock bass consumption declined in both basins during 1985–1989, reflecting changes in rock bass population abundance (Fig. 4). Estimates of *C. punctipennis* consumption by rock bass were small when compared with *C. punctipennis* production in 1992 and 1993. Rock bass consumption rates did not exceed 65 μg dry weight·m^{-3}·day^{-1} in the reference basin, except in 1985 when consumption was unusually high (101 μg dry weight·m^{-3}·day^{-1}) during mid-August. In contrast, production rates of later instar *C. punctipennis* during May, June, and August 1992 and 1993 exceeded 200 μg dry weight·m^{-3}·day^{-1} (Table 4). As a consequence of emergence and reproduction, production rates of later instar *C. punctipennis* tended to

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Fig. 2. Biomass of edible rotifer prey for first instar *C. punctipennis* in the reference (broken lines) and treatment (solid lines) basins of Little Rock Lake. Data series show the period 1 May through 31 August and diamonds indicate 15 June through 31 July samples. See Methods for a description of edible prey.

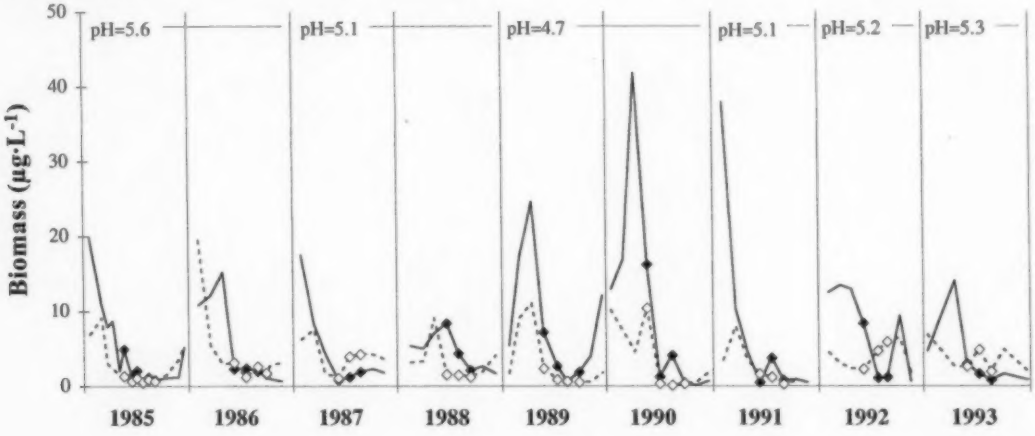
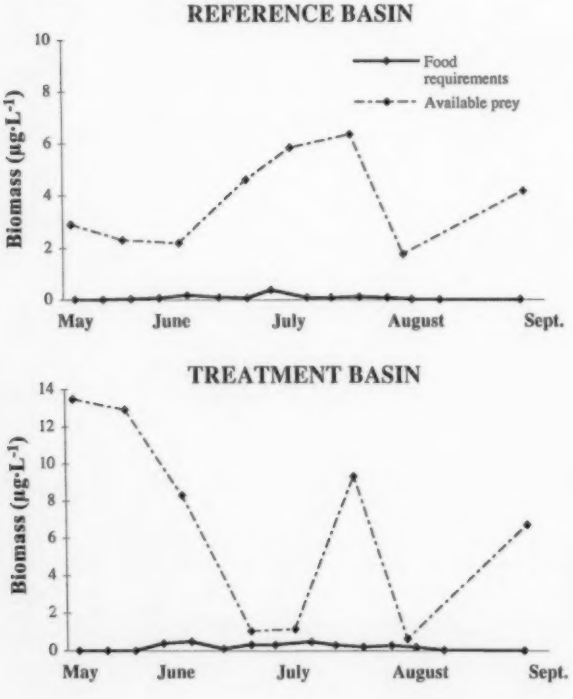


Fig. 3. Estimated food requirements of first instar C. *punctipennis* in the reference and treatment basins of Little Rock Lake during 1992. Biomass of available prey in the edible size range biomass was plotted for comparison.



decline during the month of July (Table 4). However, *C. punctipennis* production exceeded rock bass consumption by severalfold even during this period of reduced production.

Our assessment of the potential of rock bass to constrain *C. punctipennis* abundance was deliberately conservative. That is, rock bass consumption of *C. punctipennis* was overestimated by assuming that (*i*) rock bass feed exclusively on *C. punctipennis*, (*ii*) all rock bass growth occurred during the summer and early fall, and (*iii*) no rock bass mortality occurred during the growing season. Furthermore, estimates of production of later instar *C. punctipennis* during 1992 and 1993 were probably underestimates of production during previous years because *C. punctipennis* biomass was unusually low (Fig. 1). Thus, comparison of our estimates of rock bass consumption and *C. punctipennis* production suggests that rock bass abundance in Little Rock Lake (<600 adult fish per lake basin) was too low to exert strong predation pressure on *C. punctipennis*.

Invertebrate predation

Several lines of evidence suggested that invertebrate predation constrains *C. punctipennis* abundance in Little Rock Lake. Abundance of adult *M. edax* was relatively high (approximately $2 \cdot L^{-1}$) in the reference basin throughout the experiment (Fig. 5). In the treatment basin, *M. edax* abundance declined significantly with the acidification (Fig. 5, Table 3). *Mesocyclops edax* densities in midsummer are critical for evaluating *Mesocyclops–Chaoborus* interactions because this is the period of overlap between *M. edax* and early instar *C. punctipennis*. Low ($<0.06 \cdot L^{-1}$) midsummer densities of *M. edax* corresponded with unusually high *C. punctipennis* abundance in the treatment basin in 1988–1991. Overall, there was a significant negative correlation between average midsummer *M. edax* abundance (weighted by the inverse of the variance to account for differences in number of midsummer sampling dates among years; the maximum allowable weighting was set by the highest weighting in years when *M. edax* were present) and average summer *C. punctipennis* abundance ($r = -0.55$, $p < 0.02$).

Calculations based on spatial overlap of predator and prey, predator density, and laboratory-based predation rates suggest that *M. edax* are capable of exerting strong predation pressure on *C. punctipennis*. The Williamson and Stoeckel (1990) overlap index was 1.6 in the treatment basin, indicating that the

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Fig. 4. Estimated consumption of *C. punctipennis* by rock bass in the reference (broken lines) and treatment (solid lines) basins of Little Rock Lake. Data series show the full annual cycle.

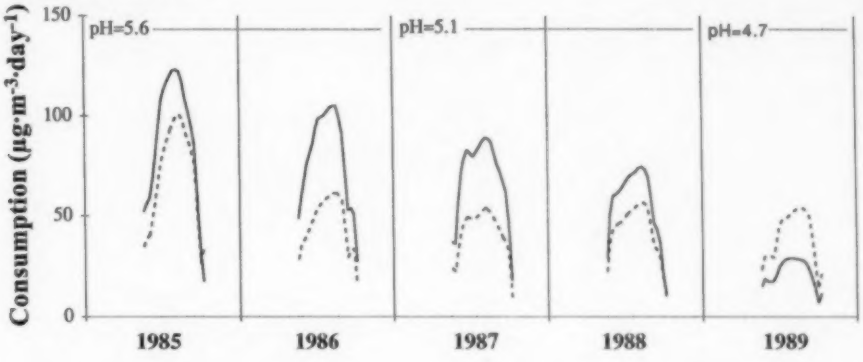

Table 4. Production rates of later instar (III and IV) *C. punctipennis* in the reference and treatment basins of Little Rock Lake.

	Reference basin		Treatment basin	
	n	Production rate (µg·m^{-3}·day^{-1})	*n*	Production rate (µg·m^{-3}·day^{-1})
May 1992	1	938.6	1	1460.9
June 1992	3	262.4±55.8	3	1561.8±1035.2
July 1992	5	186.1±39.4	5	796.6±188.7
August 1992	4	412.0±60.7	4	1628.1±384.3
May 1993	2	345.3±94.0	2	2918.9±507.6
June 1993	4	446.2±163.7	4	1613.1±507.23
July 1993	4	187.0±37.8	4	1731.1±1187.2
August 1993	5	726.4±269.7	5	5556.0±1402.3

Note: Production rates are given as the mean ± SD.

spatial distributions of *M. edax* adults and first instar *C. punctipennis* are positively correlated in Little Rock Lake. With this degree of spatial overlap, a typical *M. edax* population in the treatment basin of Little Rock Lake (0.5–3.0·L^{-1}) preying on first instars at the rate of 0.5 first instar *C. punctipennis* per day is capable of consuming 400–2400 first instar *C. punctipennis*·m^{-3}·day^{-1}. Production of first instar *C. punctipennis* was much lower than this estimated predation rate. In 1992 and 1993, daily production rates of first instars in the reference basin ranged from 6.49 to 12.78 first instars·m^{-3}·day^{-1}. Furthermore, maximum daily production rates did not exceed 248.39 first instars·m^{-3}·day^{-1}, even in 1993 when first instar production was unusually high in the treatment basin. These results strongly suggest that when *M. edax* are abundant, this invertebrate predator could create a survivorship bottleneck for first instar *Chaoborus* spp. Virtual elimination of *M. edax* from the treatment basin likely resulted in a dramatic increase in survivorship of first instar *C. punctipennis*.

The importance of invertebrate predation is further supported by the decline in *C. punctipennis* abundance that occurred following recovery of *M. edax* in the treatment basin in 1992 (Figs. 1 and 5). While *C. punctipennis* abundance also decreased in the reference basin in 1992 and 1993, the difference in *Chaoborus* abundance between basins declined substantially following the *M. edax* recovery. The average difference in *C. punctipennis* abundance (treatment minus reference) was 950 individuals/m^3 for 1988–1991 and declined to 470 individuals/m^3 in 1992–1993. Thus, both the temporal dynamics of *M. edax* in the treatment basin and our assessment of the potential predation impact of *M. edax* on first instar *C. punctipennis* suggest that invertebrate predation was an important constraint on *Chaoborus* abundance in Little Rock Lake.

Discussion

This study considered the relative importance of food limitation, fish predation, and invertebrate predation for regulation of *C. punctipennis* population dynamics in an experimentally acidified lake. Food limitation and fish predation did not appear to strongly influence *C. punctipennis* dynamics over the range of pH imposed on the treatment basin of Little Rock Lake. Rather, the dramatic increase in *C. punctipennis* abundance in the treatment basin appeared to be driven largely by a reduction in the intensity of predation on first instar *C. punctipennis* by the invertebrate predator, *M. edax*. Our results suggest that juvenile bottlenecks in stage-structured populations can be driven not only by food limitation (e.g., Neill and Peacock 1980; Neill 1988), but also by predation.

Food limitation

The relatively weak effect of food limitation on early instars in Little Rock Lake, where summer rotifer densities range from 100 to 1000·L^{-1}, is consistent with previous studies of food requirements of first instar *C. punctipennis* and other *Chaoborus* species. Fischer and Moore (1993) reported high survivorship of first instar *C. punctipennis* at rotifer densities greater than 500·L^{-1}. Other *Chaoborus* species appear to have similar food requirements during the first instar stage. In a series of experiments conducted in an ultraoligotrophic lake, first instar *Chaoborus trivattatus* were released from food limitation in treatments where rotifer densities ranged from 200 to 1000·L^{-1} (Neill and Peacock 1980; Neill 1988).

The importance of food limitation for *Chaoborus* populations appears to depend on lake trophic status. Neill and Peacock (1980) and Neill (1988) clearly documented the importance of food limitation in ultraoligotrophic lakes. In these lakes, mortality of first instars from starvation can be severe (>90%), creating a population bottleneck. Food limitation of early instars may be less important in more productive lakes. In a survey of acidified lakes across a range of lake

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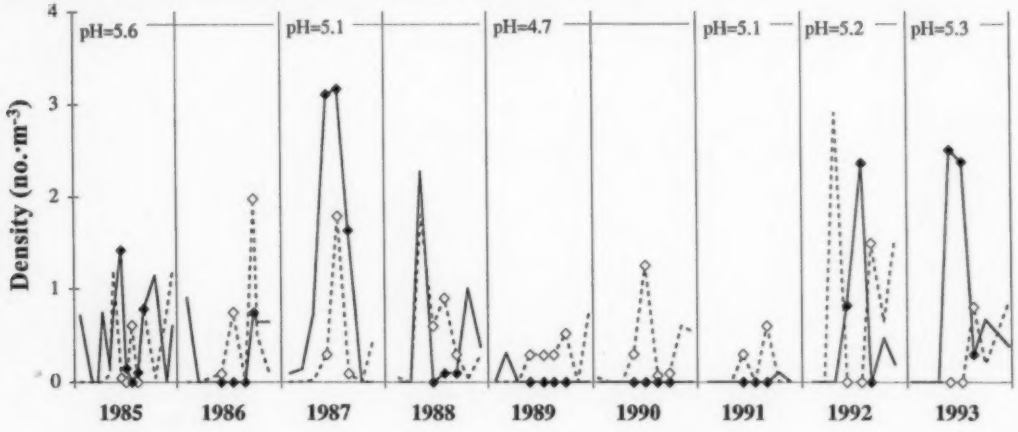
Fig. 5. Abundance of *M. edax* adults in the reference (broken lines) and treatment (solid lines) basins of Little Rock Lake. Data series show the period 1 May through 31 August, and diamonds indicate 15 June through 31 July samples.



trophy, Yan et al. (1985) reported high *C. punctipennis* abundance in lakes with higher nutrient concentrations. Prior to acidification, nutrient concentration in Little Rock Lake (total nitrogen 190 $\mu g \cdot L^{-1}$, total phosphorus 9.0 $\mu g \cdot L^{-1}$; Brezonik et al. 1986) was similar to the most productive lakes included in the Yan et al. (1985) survey. Our results suggest that food limitation is rarely an important constraint on *Chaoborus* population dynamics in systems as productive as Little Rock Lake.

Fish predation

Our conclusions regarding the role of fish predation in Little Rock Lake are not consistent with conventional wisdom on regulation of *Chaoborus* population dynamics in acidified lakes. Several studies have attributed substantial increases in *C. punctipennis* abundance to the elimination of fish populations following acidification (Eriksson et al. 1980; Yan et al. 1982, 1985). Indeed, dramatic changes in *Chaoborus* population abundance have been documented following experimental manipulation of planktivorous fish (Northcote et al. 1978; Stenson et al. 1978; Elser et al. 1987; Soranno et al. 1993). In Little Rock Lake, the numerically dominant fish species were fairly insensitive to acidification, and fish predation appeared to be relatively unimportant in controlling the increase in *C. punctipennis* abundance. Clearly, the magnitude of the change in fish abundance is critical in determining whether fish predation drives *Chaoborus* responses to acidification.

Invertebrate predation

This study is the first report of the control of *C. punctipennis* population dynamics by a common and widely distributed copepod. Other studies have suggested that predation by the copepod *Diaptomus shoshone* may exclude *Chaoborus* spp. from fishless ponds in the Rocky Mountains (Sprules 1972; Dodson 1974; Williams 1980). However, distribution of *D. shoshone* is limited to small lakes in the Rocky and Sierra Nevada mountains (Wilson 1959). In contrast, *M. edax* is abundant in lakes throughout North America (Carter et al. 1980). Thus, regulation of *Chaoborus* population dynamics by invertebrate predators may be more common than previously thought.

Future studies investigating the impacts of invertebrate predation on *Chaoborus* populations may extend the generality of our findings beyond the specific taxa found in Little Rock Lake. In addition to *M. edax* and *D. shoshone*, other large predatory copepods like *E. lacustris* are capable of consuming first instars of small-bodied *Chaoborus* species (Moore Rodenhouse 1986). The impact of these predators on *Chaoborus* populations will depend on their predation rates, as well as on the degree of spatial and temporal overlap of predator and prey. As a consequence of their small body size, *C. punctipennis* first instar larvae (1.6–2.4 mm body length; LaRow and Marzolf 1970) may be particularly vulnerable to invertebrate predators. However, invertebrate predators may also consume first instar larvae of other North American *Chaoborus* species like *Chaoborus flavicans* (1.7–3.0 mm; Parma 1971).

Chaoborus spp. occur in lakes spanning a broad range of environmental conditions (Carter et al. 1980). Our results indicate how processes regulating *Chaoborus* populations may shift across these environmental gradients. Previous studies have clearly demonstrated the importance of both fish predation (Northcote et al. 1978; Stenson et al. 1978; Elser et al. 1987; Soranno et al. 1993) and food limitation (Neill and Peacock 1980; Neill 1988) as constraints on *C. punctipennis* dynamics. Here, we suggest an additional mechanism (invertebrate predation) that may limit survivorship of small-bodied *Chaoborus* species, particularly in productive lakes where food resources for early instars are abundant. Thus, the relative importance of food limitation, vertebrate predation, and invertebrate predation for *Chaoborus* populations depends on environmental factors such as lake productivity and pH.

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Shelter sharing and chemical courtship signals in the lobster *Homarus americanus*

Paul J. Bushmann and Jelle Atema

Abstract: In a 3.7-m Y-maze flume study of social odor-mediated behavior of mature lobsters (*Homarus americanus*), females preferred male- but not female-occupied shelters over empty shelters: they detected them from a distance and spent much time trying to enter. Males did not show distant detection and preference for female shelters but did spend much time trying to enter female but not male shelters once nearby. Sheltered resident males showed strong aggression toward visiting males but only mild aggression to visiting females; intermolt and premolt females could enter resident male shelters, cohabit for at least 7 h, receive mating attempts, and sometimes mate. Premolt females or females with sperm plugs resisted mating attempts. Visiting females released more than four times and males more than seven times as much urine during a shelter approach compared with an equal time in isolation. Females with or without urine release entered male-occupied shelters successfully, but mating attempts rarely occurred when female urine release was blocked. Female urine block resulted in greatly increased resident male aggression toward her, reaching the same levels elicited by visiting males with or without urine block. The results show that female urine signals reduce male aggression and facilitate mating.

Résumé : Au cours d'une étude sur le comportement social régi par des stimuli olfactifs chez les homards à maturité, effectuée dans un labyrinthe en Y de 3,7 m, les femelles préféraient aux abris vides les abris occupés par des mâles mais non par des femelles : elles les détectaient à distance et passaient beaucoup de temps à essayer d'y entrer. On n'a pas observé chez les mâles de comportement à distance de détection et de préférence des abris des femelles; une fois à proximité, ils passaient cependant beaucoup de temps à essayer d'entrer dans les abris des femelles mais non dans ceux des autres mâles. Les mâles résidants des abris manifestaient une forte agressivité à l'égard des mâles visiteurs, mais une agressivité modérée à l'égard des femelles; les femelles en phases intermue et prémue pouvaient entrer dans les abris des mâles résidants, cohabiter avec eux pendant au moins 7 h, faire l'objet de tentatives d'accouplement, et parfois s'accoupler. Les femelles en prémue ou présentant un bouchon spermatique résistaient aux tentatives d'accouplement. Les femelles en visite libéraient plus de 4 fois plus, et les mâles plus de 7 fois plus, d'urine pendant l'approche d'un abri que pendant une période égale passée dans l'isolement. Les femelles avec ou sans émission d'urine entraient avec succès dans les abris occupés par les mâles, mais les tentatives d'accouplement avaient rarement lieu lorsque l'émission d'urine était bloquée chez les femelles. Le blocage de l'émission d'urine chez une femelle provoquait une hausse de l'agressivité du mâle résidant à l'égard de la femelle, agressivité qui atteignait le niveau suscité par la visite de mâles, avec ou sans blocage de l'émission d'urine. Les résultats montrent que les signaux constitués par l'émission d'urine chez les femelles réduisent l'agressivité chez les mâles et facilitent l'accouplement. [Traduit par la Rédaction]

Introduction

Crustaceans display a wide range of mating systems and behaviors. Some species pair only for copulation (Yano et al. 1988; Kamiguchi 1972; Lipcius et al. 1983), while others form longer lasting pair bonds. Pair bonds include mate guarding by the male (Van Engel 1958; Anstensrud 1992) or guarding linked with male occupation and defense of a shelter (Shuster and Caldwell 1989; Borowsky 1983). A shelter-based mate guarding system is used by the lobster *Homarus americanus* (Atema et al. 1979).

Shelters are important for most aspects of lobster life, including courtship and mating. Male lobsters establish and defend shelters, evicting adjacent males (Karnofsky and Price 1989; Karnofsky et al. 1989). Late-premolt females enter dominant-male mating shelters and cohabit with that male for approximately 2 weeks, during which time female molting and mating occur (Cowan and Atema 1990). Mating involves male deposition of a spermatophore in the seminal receptacle of the female, the outer portion forming a sperm plug covering the opening (Templeman 1934; Aiken and Waddy 1980). During cohabitation the male guards the shelter and the female. The female remains in the shelter until her new exoskeleton has hardened (Atema et al. 1979; Karnofsky et al. 1989). Shelter sharing has only been reported during this cohabitation mating; lobsters are otherwise thought to be solitary (Ennis 1984; Karnofsky et al. 1989).

A cohabitation mating system offers females protection during the molt, a vulnerable time in the life of a lobster. Animals molting without cohabitation have been observed blocking shelter entrances and sealing themselves in (Atema et al. 1979; Karnofsky et al. 1989). This system of mating immediately following molting also allows females to remain fertile for the greatest possible intermolt period: females with spermatophores can remain fertile for up to 3 years (Waddy and Aiken 1986), but they lose their spermatophore when they

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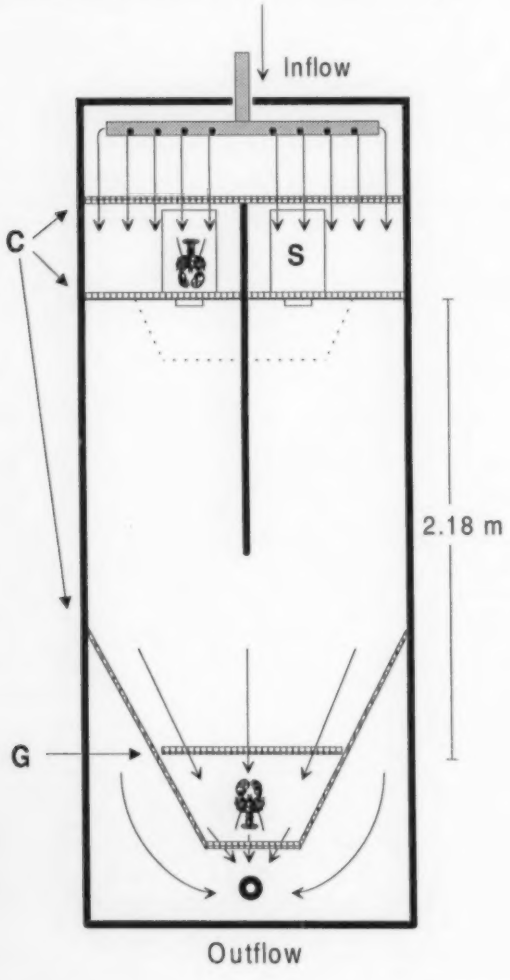
Fig. 1. Diagram of flume used for all trials. Collimators (C) are screens of 1-cm^3 mesh, used to smooth turbulent flow and direct current into the start position. Lobsters (approximately to scale) are shown in the start position and the left shelter. Arrows indicate general direction of water flow. S, artificial shelters; G, gate.



molt. In addition, cohabitation mating offers the male the important advantage of paternity insurance through mate guarding.

A less understood mating system exists involving intermolt females. Intermolt mating is thought to occur when females have molted without a mating opportunity. Such mating is of short duration and cohabitation does not occur. Females can then reproduce without waiting for the next molt period (Dunham and Skinner-Jacobs 1978; Waddy and Aiken 1990). It has been observed only in the laboratory, usually when shelter is poor or in short supply, and the natural context is unknown. However, its laboratory existence suggests that lobsters in nature may use multiple mating strategies (Waddy and Aiken 1990).

Chemical signals have been suspected to play a role in lobster mating for many years. Several workers have described male attraction to tank water of freshly molted females (Hughes and Matthiessen 1962; McLeese 1970, 1973). In addition, Atema and Engstrom (1971) demonstrated that males showed reduced aggression when exposed to the tank water of freshly molted females. Atema and Cowan (1986) proposed urine as a carrier for lobster chemical signals and Cowan (1991) showed that ablation of the lateral antennules of either a cohabiting male or female leads to abnormal mating behaviors. However, Dunham (1979) did not observe male courtship behavior with water from freshly molted females, while Snyder et al. (1993) described mating occurring while male or female urine release was blocked. Therefore, the importance of urine and other chemical signals in lobster mating remains unclear.

For this study we hypothesized that females locate male-occupied shelters from a distance and release urine signals to facilitate entry and mating. We compared female and male behaviors, with or without urine release, as they approached sheltered males and females, and the behavioral responses of the sheltered animals. We also examined the influence of female molt stage and sperm plugs on female and male behaviors. A few preliminary results of this work have been reported (Bushmann and Atema 1994).

Materials and methods

All lobsters in this study were adult, sexually mature males and females, 75–85 mm in carapace length, caught in the Woods Hole, Massachusetts, area by local lobstermen. They were kept in communal holding tanks for no longer than 3 weeks until use. Two males or two females of equal carapace length were paired and placed into one of two flow-through 1.84 × 0.51 × 0.30 m fiberglass tanks. Each tank contained one shelter. The pairs were allowed to establish a dominance relationship, evidenced by the dominant animal occupying the shelter. Pairs were allowed at least 1 week before use in an experiment. The lobsters paired in this way were termed resident males or females and were maintained throughout an experiment. Three days before an experiment other males or females were each placed individually into 1 of 10 flow-through 0.30 × 0.25 × 0.14 m plastic tanks. The lobsters placed into the individual tanks were termed visiting animals.

All tanks received unfiltered seawater from the general supply of the Marine Biological Laboratory in Woods Hole through individual seawater spigots. Outflow from the visiting animal tanks pooled and flowed collectively and equally into the tanks of the resident pairs, such that these tanks received the majority of their seawater from the individual tanks. In this way, the residents were chemically familiar with the visiting animals, while the visiting animals were not chemically familiar with the residents. The rationale for this arrangement was based upon the observations that male lobsters held in lengthy isolation exhibit increased aggression (Hoffman et al. 1975). Chemical awareness of other lobsters besides the tank mate was thought to represent a more natural condition and might prime the resident animals for mating behavior. No visual communication was possible between any tanks. All animals were housed in the same room, on a 14 h light : 10 h dark reversed phase light cycle. Experiments were performed in the dark phase, during the months of June–October of 1993 and 1994. Water temperature ranged from 15 to 24°C.

All experiments were conducted in a 3.7 × 1.4 × 0.30 m fiberglass flume, equipped with a flow-through seawater system (Fig. 1). A barrier split the upstream end of the flume into a *Y*. Two plastic containers (0.30 × 0.25 × 0.14 m) represented artificial lobster shelters and were placed at the upstream ends of each arm. Each shelter had several 1-cm upstream holes to permit water flow through the shelter and

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a downstream entrance constructed of 152-mm i.d. PVC pipe. The barrier prevented any water flow between shelters. The shelter entrances were either open or closed with two bars that prevented entry and exit but allowed visual, chemical, and tactile communication. The downstream end of the flume contained a start position 2.18 m from the shelter entrances. Seawater flowed through and around the shelters at 1 cm/s and drained through a standpipe directly behind the start position. Dye studies indicated that water flowing through the shelters pooled and mixed in the start position. Any animal in this position therefore received simultaneous chemical information from both shelters. Lobster behaviors and movements in the flume were recorded on videotape with a Panasonic WV-1850 low-light camera. The flume was illuminated by four 60-W incandescent bulbs dimmed to the lower limit of camera resolution.

A trial involved placing a resident, dominant animal in one of the shelters, while the other remained empty. Only dominants were used in this study because during cohabitation mating the dominant males are most attractive to females and are more likely to mate successfully (Atema et al. 1979; Cowan and Atema 1990). At least 1 h later, a visiting animal was placed in the start position. After 10 min acclimation, a trial began by dropping a gate allowing the visiting lobster to move freely about the flume. After 15 min the trial was ended and the visiting lobster removed. Thirty minutes were allowed between trials to clear the odor of the visiting animal. Dye tests showed complete water turnover in this time period. Each day no more than five visiting animals were tested in sequence. This would not be unusually high when compared with observations in naturalistic aquaria (Cowan and Atema 1990) and the field (Karnofsky et al. 1989).

The experiment was divided into two parts. Part 1 tested all possible combinations of males and females as visiting and resident animals to determine whether visiting animals would locate, approach, and attempt to enter resident animal shelters. Part 2 examined how urine release by visiting males and females influenced shelter entry and resident male mating behavior. Both parts followed the same general protocol, with variations as described below.

Part 1: attraction to sheltered lobsters

In this experiment, shelter entrances were barred to prevent entry or exit. Behaviors at each shelter entrance were recorded by videotape camera. Residents were switched between shelters each day to avoid bias owing to possible learning or intrinsic shelter preference. Animals were tested according to the following protocol: (*i*) one single resident male, 20 visiting females; (*ii*) one single resident male, 10 visiting males; (*iii*) one single resident female, 20 visiting females; and (*iv*) one single resident female, 10 visiting males.

Each condition was tested with different visiting and resident animals. Two parameters were examined. First approach was defined as the first shelter approached from the start position. A visiting animal crossing a line drawn 0.30 m from each shelter entrance was scored as having approached that shelter. Differences in first approach between the two shelters were evaluated using a χ^2 test. The second parameter was time spent entering. A visiting animal would often try to enter a shelter, although the bar prevented success. The time a visiting animal spent trying to enter was recorded and used as a measure of the visiting animal's motivation to enter the shelter of the resident. Differences in mean time spent entering between the two shelters were evaluated with a Mann–Whitney *U* test.

Part 2: shelter entry behavior

In this experiment a single dominant male was placed in a shelter and females ($N = 20$) or males ($N = 15$) were tested as visiting animals. Two dominant–subordinate male pairs were prepared. The shelter entrances were open, allowing entry. A low-light videotape camera (Panasonic WV-1850) was positioned over the shelters, which were fitted with 0.30×0.25 m transparent Plexiglas tops to allow observation. Shelters were screened from the flume incandescent lights and were illuminated by one red photographic safelight positioned 1 m above the shelter tops.

Visiting animals were fitted with a nephropore catheter (Lindstrom 1991). This consisted of two 1-cm pieces of 1/8-in. i.d. (1 in. = 25.4 mm) Tygon latex tubing, the end of each glued over a nephropore so that the nephropore was fully enclosed by the tubing. The glue was a thick cyanoacrylate (Zap-a-gap), applied with an accelerator (Zip Kicker). A piece of 1/16-in. i.d. PVC tubing was inserted into the distal end of each piece of latex tubing and these PVC tubes led posteriorly to a Y connector glued to the dorsal portion of the carapace. A single piece of 1/16-in. i.d. PVC tubing led to a vented collecting bottle floating on the surface. In these experiments the catheters served two purposes. They prevented urine release into the environment, testing the effects of missing urine signals on the behaviors observed. By collecting urine they allowed measurement of urine release volume and the correlation of release rates with behavioral observations.

All males and females were tested with catheters in open and closed condition. In closed condition the catheters were fully connected. In open condition the PVC tubing between the Tygon tubing and the Y connector was removed, allowing normal urine release. Fifty percent of the visiting animals were tested in random sequence first with open, then with closed catheters, and the order was reversed for the remaining animals. The resident male was moved to the other shelter after every five trials to avoid shelter bias. Visiting females were molt staged by examination of pleopod tips for the formation of new cuticle (Aiken 1973) and checked before and after experimentation for the presence of a sperm plug in the seminal receptacle (Waddy and Aiken 1991).

Lobster aggression (Scrivener 1971), mating (Herrick 1896; Templeman 1934; Hughes and Matthiessen 1962; Atema and Engstrom 1971), and courtship (Atema et al. 1979; Atema and Cobb 1980; Cowan and Atema 1990) have been well documented. The behaviors used in this study were adapted from a recent review (Atema and Voigt 1995). Entering, eviction, and mating behavior were summed for all trials and compared between males and females and between open and closed catheter conditions using a χ^2 test. For those trials in which successful entering occurred, aggressive behavior by the resident toward visiting animals was recorded. These aggressive behaviors were summed for all trials and compared between males and females and open and closed catheter conditions using a Wilcoxon signed-rank test.

Behaviours used in the analysis are summarized in Table 1.

Results

Part 1: attraction to sheltered lobsters

Of all permutations tested, only the one in which females visited resident males produced both significant nonrandom first approach (Fig. 2A) and time spent entering (Fig. 2B). Visiting females showed a nonsignificant trend for approaching resident female shelters but rarely attempted to enter. Males did not display any ability to locate sheltered females but did show significantly nonrandom entering attempts once the shelter was encountered (Fig. 2B). Males showed no trend for approaching or entering resident male shelters.

Part 2: shelter entry behavior

Visiting female, visiting male, and resident male behavioral outcomes are compared in Table 2. Both females and males attempted to enter resident male shelters with no significant difference between female and male success rates. However, in all but one case successful male entry required eviction of the resident. Eviction success was correlated with visiting male

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Table 1. Summary and definition of behaviors used in analysis.

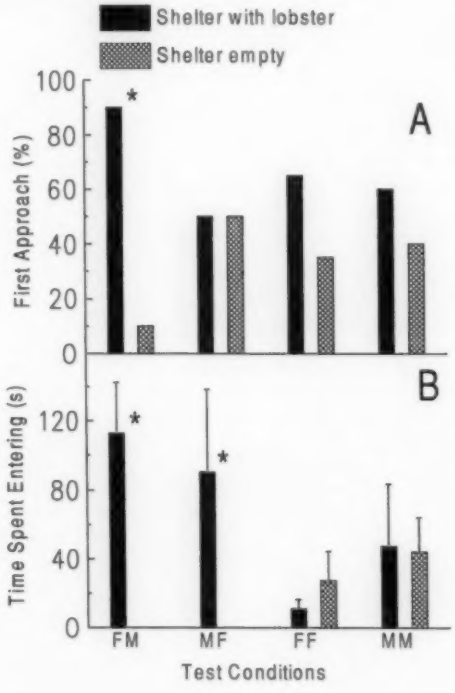
Term	Definition
Entering	Visiting animal pushes one or both chelae fully into the shelter entrance
Successful entering	Visiting animal enters shelter completely such that uropods and claws are fully within the shelter cavity
Eviction	Resident animal moves completely out of shelter
Attempted mating	Resident male is above female, slightly behind and parallel to her; grasping the edge of her carapace with walking legs and lifting it
Successful mating	Female is upside down, claws forward and abdomen outstretched; male is parallel to and above her; confirmation of success required examination of the female for a new sperm plug after mating
Push	Pressing chelae against opponent animal and walking forward, with or without swimmeret current
Claw lock	Clamping of chelae onto opponent's chelae, either inside the open chelae or on the outside edge, or onto another body part
Scissor	Bringing the chelae from a laterally extended position rapidly inward in a scissoring motion directed toward opponent; may touch opponent in the process
Snap	Closing chelae quickly, directed toward opponent's body; may or may not actually seize opponent

carapace length (Spearman's $r = 0.69$, $p < 0.05$). In contrast, entering females never evicted the resident; instead, the resident male and visiting female shared the shelter for the duration of the trial.

The resident male behaved differently toward visiting females and males. In 10 of 14 successful shelter entries the resident attempted to mate with the female and was successful in 4 trials. Visiting males never received resident male mating attempts. Females that entered but received no mating attempt from the resident male were nonetheless allowed to remain in the shelter with little aggression directed toward them. Visiting males evoked aggressive behaviors from the resident male. These consisted mainly of pushing, with bouts of claw lock, scissor, and snap (Fig. 3). The males usually remained facing each other, pushing until the visiting animal was repulsed or gained entry into the shelter. The latter usually signaled eviction of the resident, who then abandoned the shelter. Female shelter entry began similarly, but the resident male responses were less aggressive, showing significantly fewer bouts of pushing and high-level aggression (Fig. 3). Once a female had gained entry, she always turned, presenting her abdomen to the male, and often flattened herself along one wall of the shelter, claws forward and outstretched. The resident male exhibited little aggression toward her at this point, and either attempted to mate or did not interact with her for the duration of the trial.

All females were molt staged and examined for the presence of a sperm plug before and after experimentation (Table 3). Females were grouped as either intermolt (stage C; 10 females) or premolt (stage D_0 or D_1; 10 females). No females of later molt stage were collected for use during this study. Seven females possessed sperm plugs, while 13 did not. Molt stage or sperm plug presence did not affect female

Fig. 2. (A) Shelter first approached by visiting males or females with resident males or females. Astersisks indicate statistically significant differences between shelters. FM, visiting female – resident male, $\chi^2 = 12.80$, $p < 0.05$; MF, visiting male – resident female; FF, visiting female – resident female; MM, visiting male – resident male. (B) Mean time spent by visiting males or females attempting to enter shelters. Error bars are 1 SE. Asterisks indicate statistically significant differences between shelters. FM, Mann–Whitney U test, $z = 3.75$, $p < 0.05$; MF, Mann–Whitney U test, $z = 2.02$, $p < 0.05$.



attempted or successful entry. Resident male mating attempts were not influenced by molt stage or significantly by sperm plug presence, although there was a trend toward females without sperm plugs. All eight entering females without sperm plugs received mating attempts (Table 3). Females could resist mating attempts by spreading their chelae to prevent being rolled over. Premolt females and intermolt females with sperm plugs resisted mating, such that the four successful matings all involved intermolt (stage C) females without sperm plugs. Any other combination of female molt stage or sperm plug presence resulted in either no male solicitation or active female resistance. Resisting females did not mate during the trial period.

Visiting females and males were found to release significantly more urine during a 15-min trial, compared with that collected during a 15-min pretrial period in isolation (Fig. 4). Urine release by visiting females influenced resident male behaviors. Female urine block (catheters closed) did not affect the frequency of successful entry but did decrease the likelihood of mating attempts by the resident male (Fig. 5). Closing catheters also increased the frequency of occurrence of resident male aggression toward the visiting female. Pushing, the

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Table 2. Behavioral outcomes for 20 females and 15 males allowed to approach and attempt to enter the shelter of a dominant male.

	Females ($N = 20$)		Males ($N = 15$)	
Outcome	Sum	%	Sum	%
Entering behavior				
Attempted entry	16	80	11	73
Successful entry	14	88	7	64
Resident eviction	0*	0	6*	86
Mating behavior				
Attempted mating	10*	71	0*	0
Successful mating	4	40	0	0

Note: The number of trials resulting in a particular behavioral outcome is presented, both as a sum and as a percentage of the trials in which the behavior occurred. For those animals that entered successfully, resident eviction and attempted mating were compared between females and males. Asterisks indicate statistically significant differences. Eviction: $\chi^2 = 12.2$, $p < 0.05$; attempted mating: $\chi^2 = 4.9$, $p < 0.05$.

most commonly observed behavior, as well as the more aggressive behaviors claw lock, scissor, and snapping were all elevated when female urine was blocked (Fig. 5).

Closing visiting male catheters, however, produced no significant differences. The occurrences of successful entry (seven) or eviction (six) when visiting male catheters were open were not significantly different from the closed-catheter condition (seven and seven, respectively). Resident male aggression toward open-catheter visiting males (16 counts of push; 17 counts of claw lock, scissor, or snap) also did not differ significantly from aggression elicited by closed-catheter visiting males (25 counts of push; 14 counts of claw lock, scissor, or snap). These aggression levels matched closely the behavior toward urine-blocked females (Fig. 5).

Discussion

In this study, intermolt and premolt female lobsters located male shelters from a distance of over 2 m and successfully entered with only mild resident male aggression. Although females had an overall tendency to approach any occupied shelter, they significantly preferred to approach and enter only male shelters (Fig. 2). Female urine release did not influence her shelter entry, but once she was inside it reduced resident male aggression and elicited male mating attempts (Fig. 5). Visiting males did not demonstrate a preference for locating sheltered animals, but they did spend much time attempting to enter female shelters (Fig. 2). Resident males responded to visiting male entering attempts with strong aggression (Fig. 3). Urine release by visiting males, although elevated during a visit (Fig. 4), did not influence entering success or the aggressive responses of the resident males.

Visiting animals were used only once per day and at most four times in all, while the two resident males were used repeatedly throughout the experiment. In the field (Karnofsky et al. 1989) and in large aquaria (Cowan and Atema 1990) all lobsters, and dominant males in particular, are visited regularly in their shelters. Repeated use of a single resident permitted more comparable observations of visiting animal behavior and had no discernible effect upon resident male behavior. The occurrences of attempted and successful male matings were not correlated with trial order during a day or with day order

Fig. 3. Summed occurrence of resident male aggressive behaviors for all trials for visiting open-catheter females and males. Behaviors are defined in Table 1 and were compared between females and males. Push, Wilcoxon signed-rank test, $z = 2.10$, $p < 0.05$; claw lock, scissor, snap, Wilcoxon signed-rank test, $z = 2.20$, $p < 0.05$.

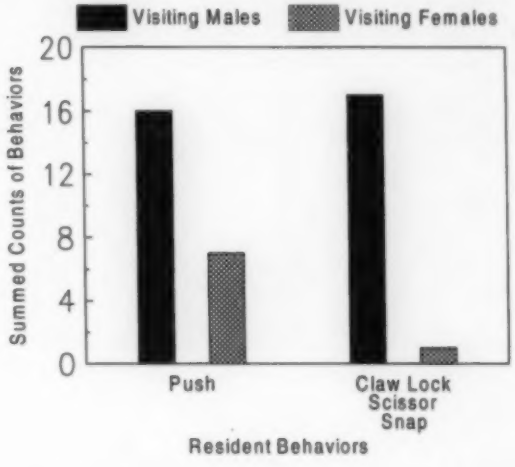

Table 3. Female molt stage and the presence of sperm plugs and their effects upon visiting female and resident male behavior.

	Female condition ($N = 20$)			
	Intermolt	Premolt	Sperm plug	No sperm plug
Female totals	10	10	7	13
Female behavior				
Attempted entry	9	7	7	9
Successful entry	8	6	6	8
Male behavior				
Attempted mating	6	4	2	8
Successful mating	4	0	0	4

Note: Values are the number of females performing or eliciting a behavior.

during the experiment. Similarly, visiting male eviction success was related only to visiting male carapace length. However, with a small resident male sample size, conclusions concerning resident male behavior must be drawn with caution.

This study suggests that intermolt mating, observed previously without the provision of shelters of sufficient size for mating (Dunham and Skinner-Jacobs 1978; Waddy and Aiken 1990), may normally occur inside shelters. Lobsters are considered to be solitary and agonistic toward conspecifics (Ennis 1984; Karnofsky et al. 1989; Steneck 1991), and shelter sharing has not been observed in the field outside of cohabitation mating (Atema et al. 1979; Karnofsky et al. 1989). However, if intermolt mating occurs inside shelters soon after entry and is not accompanied by prolonged cohabitation, it would be difficult to observe in nature. Visiting animals in this study were unfamiliar with the resident animals and their immediate surroundings. These conditions may not be typical for lobsters in stable populations (Karnofsky et al. 1989) but may be similar to those experienced by transient animals, including females

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Fig. 4. Mean urine release rates by visiting females and males with closed catheters during a 15-min trial and a 15-min pretrial isolation period. Error bars are 1 SE. Rates were compared between pretrial and trial conditions. Visiting females, Mann–Whitney U test, $z = 2.56$, $p < 0.05$; visiting males, Mann–Whitney U test, $z = 2.24$, $p < 0.05$.

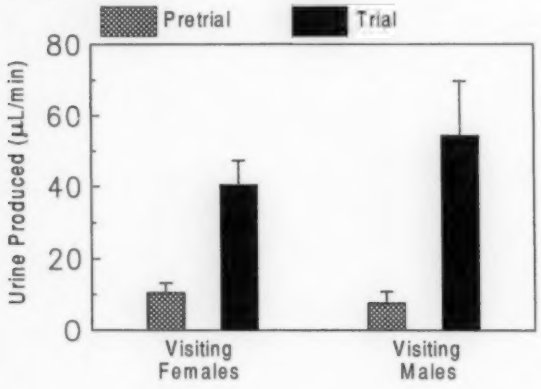
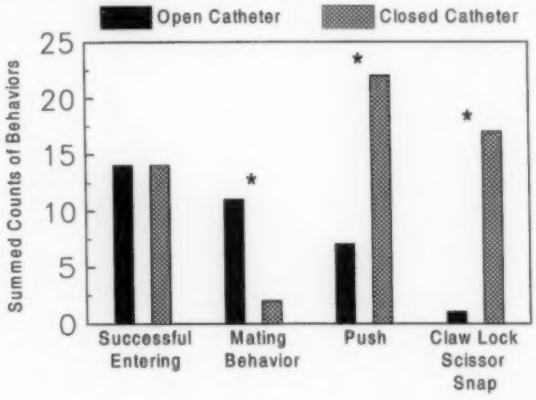


Fig. 5. Summed occurrence of successful female entering and resident male mating and aggressive behaviors for visiting females with open or closed catheters. Asterisks indicate statistically significant differences between open and closed catheter conditions. Mating behavior, $\chi^2 = 5.20$, $p < 0.05$; push, Wilcoxon signed-rank test, $z = 2.24$, $p < 0.05$; claw lock, scissor, snap, Wilcoxon signed-rank test, $z = 2.31$, $p < 0.05$.



during migration (Campbell 1986; Pezzack and Duggan 1986). It may be useful for field workers to examine these migratory events for evidence of intermolt mating or shelter sharing.

Females with sperm plugs and in premolt resisted mating attempts yet successfully entered and remained in the resident male shelters (Table 3). Shelter sharing with these females precluded resident male mating during that period. This apparent male cost suggests that prior to shelter entry, males cannot determine female receptivity, either through molt stage or sperm plugs. By not providing this information, females gain access to shelter, a phenomenon described in stomatopod crustaceans (Caldwell 1986). Males could still benefit overall if receptive intermolt females occasionally entered and mated with the resident. Field observations of intermolt female entry into male shelters would provide evidence for the existence of intermolt mating in nature. Without any mating possibility it is difficult to see why males would allow intermolt females to enter and remain in their shelters.

Because females were removed at the end of 15-min trials the full duration of shelter sharing was not measured. In a preliminary test, four females were allowed to remain in the male shelter after entry for 2, 3, or 7 h. All females and resident males were still in the shelter at the end of each period. If a female entering at night remained until daylight, shelter sharing would likely last at least until the following night, as lobsters are unlikely at least in shallow water to leave shelters during the day (Karnofsky et al. 1989).

Female chemical signals carried in urine did not influence shelter entry, but after entry female urine reduced resident male aggression and increased the likelihood of male mating attempts (Fig. 5). Indeed, resident males treated urine-blocked females similarly to males with or without urine. These results confirm earlier reports (Atema and Engstrom 1971; McLeese 1973; Atema and Cowan 1986) but appear to be at odds with some recent work in which normal mating was observed in catheterized lobsters (Snyder et al. 1993). However, in the latter study animals were tested in open arenas, with no shelters provided. There is considerable evidence from the laboratory and field to suggest that shelters are important for lobster survival and much of lobster behavior (Scarratt 1968; Cobb 1971; Atema et al. 1979; Karnofsky et al. 1989). Lobster behavior is quite context specific, and often a stimulus will not generate a response unless the behavioral context is correct (Atema and Cowan 1986). Effects of female urine release upon male aggression and mating were seen here inside the shelter of a dominant resident male, but these effects may not be expressed in other contexts. Conversely, the observation of visiting male urine having no effect on resident male behavior does not imply that male urine has no effect upon male lobsters in other contexts, such as boxing matches outside of shelters (Karavanich and Atema 1991; Breithaupt and Atema 1993).

Although loss of female urine signals through catheter closure resulted in elevated male aggression, closed-catheter females were able to remain in the male shelter. Moreover, males with closed catheters still invoked high resident male aggression. Clearly the resident males were receiving information in addition to urine signals that influenced their reactions to visiting females and males. One likely source of information was the posture of the visiting animal. Females always adopted a crouching posture and turned around with their claws facing away from the male, as described in Atema et al. (1979). This female posture resulted in less resident male aggression. Visiting males faced the resident males, spreading their chelae and (or) raising their bodies from the substrate. This may have evoked resident male shelter defense behavior regardless of any urine signal. The single male that entered and remained in a resident male's shelter without eviction immediately adopted the crouching posture exhibited by females and retained it for the duration of the trial.

This study provides evidence that female urine signals play an essential role in facilitating intermolt mating. Loss of chemical signal reception in resident males through antennule ablation has been shown to lead to increased female injury during cohabitation mating (Cowan 1991). Together, these

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studies suggest that lobsters rely heavily on chemical communication during courtship and mating. This is not surprising in an almost exclusively nocturnal animal. Therefore, lobsters may be vulnerable to even low levels of chemical pollution if those chemicals disrupt signal reception by damaging the antennular chemoreceptors or if they mimic or mask the biological signal (Atema et al. 1973). Understanding the role of chemical signaling in lobster social behavior and the interactions of chemical and other sensory signals is important for accurate predictions of lobster mating success and population dynamics.

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Does intense fishing on males impair mating success of female Dungeness crabs?

David G. Hankin, Terrance H. Butler, Paul W. Wild, and Qian-Li Xue

Abstract: Commercial capture of female Dungeness crabs, *Cancer magister*, is prohibited and minimum size limits for commercial harvest of male crabs are designed to allow most males to mate at least once before capture. Annual exploitation rates often exceed 90%, however, and the resulting scarcity of large males might reduce mating success among large females. We present new data regarding (*i*) sizes of male and female crabs collected in premating embraces, (*ii*) carapace width frequencies of female Dungeness crabs, (*iii*) presence of sperm plugs and sperm, and (*iv*) fecundity. Minimum carapace width of hard-shelled mating males typically exceeds postmolt carapace width of soft-shelled females (*i*), but female Dungeness crabs exceeding the minimum legal size of males usually account for less than 5% of mature adult female crabs (*ii*), and sublegal-sized males actively participate in mating (*i*). Remnants of sperm plugs, definitive indicators of mating, were found in 97.5% of recently molted large females (*iii*), suggesting that virtually all molting females mate regardless of size. On the basis of (*ii*) and (*iv*), hypothetical worst-case calculations, assuming that no large females could find mates, suggest that total egg production would be reduced by no more than 2–25% among molting female crabs.

Résumé : La capture commerciale des femelles de crabe dormeur, *Cancer magister*, est interdite, et l'imposition d'une taille minimale pour la capture commerciale des crabes mâles est destinée à permettre à la plupart des mâles de s'accoupler au moins une fois avant la prise. Toutefois, les taux annuels d'exploitation dépassent souvent 90%, et la rareté de mâles de grande taille qui en résulte pourrait réduire le succès de l'accouplement chez les femelles de grande taille. Nous présentons de nouvelles données concernant (*i*) la taille des crabes mâles et femelles capturés pendant l'accolement précopulatoire, (*ii*) les fréquences des largeurs de carapace des femelles de crabe dormeur, (*iii*) la présence de bouchons spermatiques et de sperme et (*iv*) la fécondité. La largeur minimale des mâles à carapace dure qui s'accouplent dépasse généralement la largeur postmue des femelles à carapace molle (*i*), mais les femelles dont la taille dépasse la taille minimale légale des mâles représentent généralement moins de 5% des femelles adultes matures (*ii*), et les mâles de taille inférieure à la taille légale participent activement à l'accouplement (*i*). Des restes de bouchons spermatiques, qui indiquent avec certitude que l'accouplement a eu lieu, ont été observés chez 97,5% des femelles de grande taille ayant mué récemment (*iii*), ce qui permet de penser que presque toutes les femelles en mue s'accouplent quelle que soit leur taille. À partir de (*ii*) et (*iv*), des calculs hypothétiques de la pire éventualité, supposant qu'aucune femelle de grande taille ne peut trouver de partenaire, nous permet de penser que, parmi les femelles en mue, la réduction de la production totale d'oeufs ne dépasserait pas 2–25%.
[Traduit par la Rédaction]

Introduction

The Dungeness crab, *Cancer magister*, supports important commercial fisheries in California, Oregon, Washington, British Columbia, and Alaska. Commercial fisheries are managed on the basis of sex, size, and season, as are many other important crustacean fisheries (Cobb and Caddy 1989). Commercial harvest of females is prohibited, minimum size limits prohibit harvest of immature males, and fisheries are generally closed for several months encompassing the molting season for males. These three regulations are intended to (*i*) protect the reproductive potential of a fishable stock, (*ii*) allow most males to mate at least once prior to fishery vulnerability, (*iii*) minimize handling mortality for soft-shelled male crabs, and (*iv*) reduce commercial sale of male crabs that are in poor market condition (i.e., crabs that have low meat recovery).

Other fishery regulations include physical gear requirements (including (*i*) use of biodegradable releases, to prevent lost traps from continuing to fish, and (*ii*) escape ports, to allow most female and undersized male crabs to escape traps) and restrictions on numbers of traps fished by individual fishers (Alaska); limited entry schemes are developing in most coastal areas. Nevertheless, there are no direct limitations on total commercial catches of male Dungeness crabs in any of these Pacific Coast fisheries. As a consequence, exploitation rates (with respect to initial abundance of legal male crabs) in at least the northern California fishery (Gotshall 1978; Methot and Botsford 1982; Hankin 1985) and certain British Columbia fisheries (Smith and Jamieson 1989) have exceeded 90% in some years. Exploitation rates in Oregon and Washington Dungeness crab fisheries probably also reach such extreme values, but we are not aware of published estimates of exploitation rates for those fisheries.

In northern California, molting of female Dungeness crabs

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and mating of soft-shelled females with hard-shelled males takes place primarily from mid-February through mid-May, with a typical peak during early April. Male crabs, believed to be polygynous, apparently can sense when molting of female crabs is imminent, and they grasp females in a premating embrace several days prior to female molting (Butler 1960); when females molt, males grasp females in the true mating embrace at which time copulation takes place (Snow and Nielsen 1966). Following copulation, males grasp females in a postmating embrace, which may last up to 2 days (Snow and Nielsen 1966; D.G. Hankin, unpublished observations). On average, the total duration of mating activities for a single pair (from initiation of the premating embrace through completion of the postmating embrace) probably ranges from 1 to 2 weeks, a relatively short period when compared with the approximately 3-month length of the female molting season. There is thus ample opportunity for individual males to mate with many different females. Molting of male crabs usually begins in late July following completion of the mating season, with a peak during late August. From late August through October, female crabs can often be found buried in sand in shallow surf zone areas in northern California. Burial in sand is required for successful adhesion of eggs to pleopods when eggs are extruded and fertilized during late fall (Wild 1980, 1983; P.W. Wild, unpublished observations; D.G. Hankin, unpublished observations). Ovigerous females brood eggs externally until they hatch, usually from mid-December through late January. In British Columbia and Alaska, the molting and mating cycle occurs later in the year, and molting of males seems generally to precede molting of females (Shirley and Shirley 1988).

Hankin et al. (1989) and Mohr and Hankin (1989) found that annual molting probabilities exceeded 90% for adult female crabs less than 135 mm carapace width (CW), but then dropped rapidly to near zero for CW exceeding 155 mm. Although very large (>155 mm) female Dungeness crabs did not often molt, Hankin et al. (1989) provided tag recovery data confirming that these large crabs could retain viable sperm for at least 2.5 years and extrude successive egg masses without annual molting and mating. Size-specific fecundities of such skip-molt crabs that had not molted were reduced, however, when compared with size-specific fecundities of crabs that had molted.

By regulation, the northern California fishery runs from 1 December through 15 July. Since the 1980s, more than 80% of a season's landings have been taken within 6 weeks of the fishery opening (Warner 1985); most legal-sized male Dungeness crabs have usually been captured prior to peak molting of female crabs. The minimum CW for commercial capture of males is approximately 159 mm in northern California, Oregon, and Washington; approximately 154 mm in British Columbia; and approximately 165 mm in Alaska. CW measurements exclude the posterolateral spines.

Noting that exploitation rates are extremely high in many Dungeness crab fisheries, Smith and Jamieson (1991, see also Smith 1988) proposed that "many legal-sized males are caught by the commercial fishery before they have an opportunity to mate" and that, as a consequence, there might be a "loss of population egg production in intensive fisheries . . . ." Indeed, relying on male mating mark data (Smith and Jamieson 1991, Figs. 1 and 2) and sizes of males and females measured from premating embraces (their Fig. 3), they proposed that "females larger than 140 mm CW could have difficulty finding a mate if legal-sized males were rare." In northern California, at least, there seems little question that legal-sized males are relatively rare, compared with sublegal-sized males, when mating takes place.

In this paper we critically examine the evidence regarding whether or not intensive fisheries on male Dungeness crabs impair mating success and (or) limit production of eggs among female Dungeness crabs. We present new and (or) previously unpublished data from northern California and British Columbia concerning (*i*) sizes of male and female crabs collected in premating embraces, (*ii*) CW frequencies of female Dungeness crabs collected in traps nonselective with respect to female size, (*iii*) relationships among fecundity, CW, and molting history, and (*iv*) presence of sperm plugs and sperm among females of varied sizes and molting histories. We also review relevant published studies concerning reproduction and growth of female Dungeness crabs.

Although the information, analyses, and conclusions that we present are specific to populations of Dungeness crabs in the northeast Pacific, we believe that the general approaches that we have taken may be applicable in other contexts where intense harvest of male crabs (or lobsters) may give rise to concerns regarding mating success and (or) egg production among female crabs (or lobsters) (e.g., for Tanner crab see Paul 1984; Paul and Paul 1990*a*; Paul et al. 1995; for king crab see Powell et al. 1974; Paul and Paul 1990*b*; for snow crab see Sainte-Marie and Carriere 1995; for blue crab see Wenner 1989). We recognize, of course, that maintenance of population egg production is not, by itself, sufficient to guarantee adequate recruitment; recruitment may be profoundly affected by complex oceanographic phenomena independent of egg production (e.g., McConnaughey et al. 1992). Nevertheless, consistent strong recruitment would seem unlikely if egg production were substantially reduced through intense fishing on male crabs.

Materials and methods

Carapace width measurements of adult female crabs

We used experimental traps to collect adult female Dungeness crabs in northern California, relying on a contracted commercial crab fisherman to set gear and pull traps, etc. Experimental traps were modified commercial traps with reduced mesh size, closed escape ports, and six narrow triggers on each entrance tunnel; the design and performance of the experimental traps was described in detail by Diamond and Hankin (1985). Traps were nonselective with respect to size for female crabs with CW exceeding 85 mm (excluding posterolateral spines). Because most mature female Dungeness crabs have postmolt CW exceeding 110 mm, traps were nonselective with respect to the full size spectrum of adult female crabs.

Traps were set during two periods (mid-June and again in mid-July, following completion of the female molting season) at two locations (off Clam Beach, 41°00′N, and off Big Lagoon, 41°10′N) along depth contour lines at two depths (14 and 28 m) during 1992, 1993, and 1995. Traps were set at approximately 14 m off Clam Beach only during mid-July of 1994. Traps were typically baited with rockfish carcasses and squid and were allowed to soak from 2 to 6 days prior to pulling. Numbers of adult female Dungeness crabs per trap generally ranged from 30 to 90; male crabs typically made up less than 2–5% of total catches. The molting history of female crabs with respect to the previous molting season was classified into one of five categories on the basis of visual assessment of shell condition (see

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Hankin et al. 1989; Mohr and Hankin 1989): (1) definitely molted, (2) probably molted, (2–3) uncertain molting history, (3) probably did not molt, and (4) definitely did not molt.

We contrasted these recent CW frequencies of northern California female Dungeness crabs collected from 1992 to 1995 with those for (*i*) crabs collected (using our experimental traps) in northern California during 1982 and 1983 (Diamond 1983) and (*ii*) crabs collected by T.H. Butler (previously unpublished data) using closed-port commercial crab traps set at approximately 5–11 m off Roberts Bank near the mouth of the Fraser River in British Columbia during 1969 and 1970. Butler's traps were baited with squid and lifted daily. Butler's original measurements of CW included the posterolateral spines. Wherever feasible, we converted these original measurements to measurements excluding spines (conversion factor = 0.95) so as to make measurements from northern California and British Columbia directly comparable.

Carapace widths of male and female crabs in premating embraces

CW measurements (to the nearest 0.1 mm) of all male and female Dungeness crabs that were found paired in premating embraces in commercial traps were made by a contracted commercial fisherman from February through March of 1992, from February through April of 1993, and from February through May of 1995. Measurements for 1992 ceased prior to completion of mating activity, whereas measurements for 1993 and 1995 were made from all mating pairs observed throughout the commercial fishing seasons in those years. In 1992, 411 premating embrace pairs were measured; 178 premating embrace female crabs were separated from their embraces and brought to the Humboldt State University Marine Laboratory in Trinidad, California. They were then held in circulating seawater (without feeding) until they molted (usually within 1–4 days), thus generating postmolt CW of female crabs. In 1993, 101 premating embrace pairs were measured; 83 female crabs were separated from these pairs and then held in seawater until molting. In 1995, 214 premating embrace pairs were measured; 155 female crabs were separated from these pairs and then held in seawater until molting. Postmolt CW of molted female crabs was measured about 4–5 days after molting, by which time carapaces were sufficiently firm to generate reliable width measurements.

Postmolt CW measures the actual size of a female when copulation takes place. Postmolt sizes are also directly comparable with previously described female CW frequency data (collected shortly after completion of the female molting season). We therefore plotted CW of male crabs found in premating embraces against (*i*) observed premolt CW of female crabs and (*ii*) observed and (or) predicted postmolt CW of female crabs. We constructed similar graphs for an additional set of 92 paired measurements of male and female crabs found in premating embraces by T.H. Butler during the 1950s (Smith and Jamieson 1991). These graphs were used to establish the degree to which size of mating males exceeds size of females. We also plotted mean premolt widths of females found in premating embraces against 10-day periods of collection to determine if there were any trends in average female size over the course of the 1992, 1993, and 1995 mating seasons.

Examination of females for presence of sperm plugs and sperm

In June 1995, we examined 587 female Dungeness crabs captured in nonselective traps for the presence of sperm plugs and sperm. Prior molting history was judged from visual inspection of shell condition, and shell hardness was measured for each female using a durometer specifically designed for use with Dungeness crab (model 307LCRB4, Pacific Transducer Corp., Los Angeles, Calif.). Shell classifications of these crabs were made prior to dissections and were unknown to those carrying out dissections so as to minimize potential classification bias owing to sperm plug or sperm presence assessments. Durometer measurements were taken on the ventral surface of crabs midway between the notch of the posterolateral spine and the coxa (shoulder) of the cheliped; lower values correspond to softer shells. Determination of sperm plug presence required dissection and removal of the reproductive tract because sperm plugs are essentially an internal structure in Dungeness crabs (Fig. 1); the proximal sperm plug does not typically protrude from the vulva (Jensen et al. 1996; D.G. Hankin, unpublished observation), in contrast to other *Cancer* species. Paired reproductive tracts were dissected, including a section of exoskeleton around the vulva, the vagina, and a small portion of the ovary including the spermatheca. Spermathecae were examined visually for the presence of sperm material. Presence of sperm plugs was classified into one of four categories: (1) complete plug visible, (2) incomplete plug visible, (3) remnant plug visible, and (4) no portion of plug visible. Sperm presence in spermathecae was rated on a scale of 1 (swollen and full) to 4 (none present) on the basis of observation of whitish material in spermathecae. In a large subsample of these females, we dissected spermathecae, scraped spermathecae linings, and examined scrapings under a compound microscope. Presence of three- and four-rayed nonmotile spermatozoa, as described by Fasten (1918) and Langreth (1969), was verified in all crabs in the subsample.

Fecundities

We collected 100 ovigerous female Dungeness crabs from commercial traps fished near Trinidad, California, from 6 to 23 January 1992. These crabs carried late-stage (dark orange) but unhatched egg masses. CW was measured at collection (±0.1 mm) and prior molting history (with respect to the most recent molting season) was classified on the basis of visual inspection of carapace condition. We also collected 66 female Dungeness crabs by hand from the surf zone at Gold Bluffs Beach near Orick, California, 41°18′N, from 21 August through 8 October 1992. These females were taken to the Humboldt State University Marine Laboratory in Trinidad, California, where they were provided with a sand substrate (No. 3 Monterey blasting sand) and maintained in circulating seawater until they extruded eggs 2–80 days later (mean 38.5 days). At collection, CW was measured and molting history was visually assessed in the same manner as for 1992 fecundity samples. Because eggs from many of these females would have hatched during early January through mid-February of 1993, we refer to these fall 1992 fecundity samples as 1993 samples to distinguish them from samples collected during January of 1992.

At the time of collection (1992 samples) or at extrusion (1993 samples), egg masses were removed from females and transferred to Bouin's fluid for preservation for at least 1 month prior to cleaning, washing, and drying protocols that produced a fairly uniform mass of dried and hardened eggs. For 1992 samples, fecundity was estimated for 70 samples by extrapolation to total dried egg mass from mean counts made in eight replicate subsamples of 0.020 g; fecundities for the remaining 30 samples were predicted from the weight of the dried egg mass using a regression of subsample estimates of fecundity against the weights of the dried egg mass. For 1993 samples, 66 fecundity estimates were all derived from subsampling. Full details of protocols used to process egg masses and estimate fecundity were presented by Hankin et al. (1989). Dummy variable multiple regression methods were used to establish dependence of log fecundity on log CW and molting history (Draper and Smith 1981). The full regression model used for analysis was

$$(1) \qquad \log Y = B_0 + B_1 \log X + D_0 Z + D_1 Z \log X$$

where Y is estimated fecundity, X is CW, and Z is a dummy variable with value 1 for nonmolted crabs (those classified as categories 3 or 4) or 0 for molted crabs (those classified as categories 1 or 2). Crabs classified as of uncertain molting history were excluded from these regression analyses. Regression model parameters (B_1, D_0, and D_1) were judged significant when F tests rejected null hypotheses at $\alpha = 0.05$. We also reanalyzed and replotted 1984 northern California

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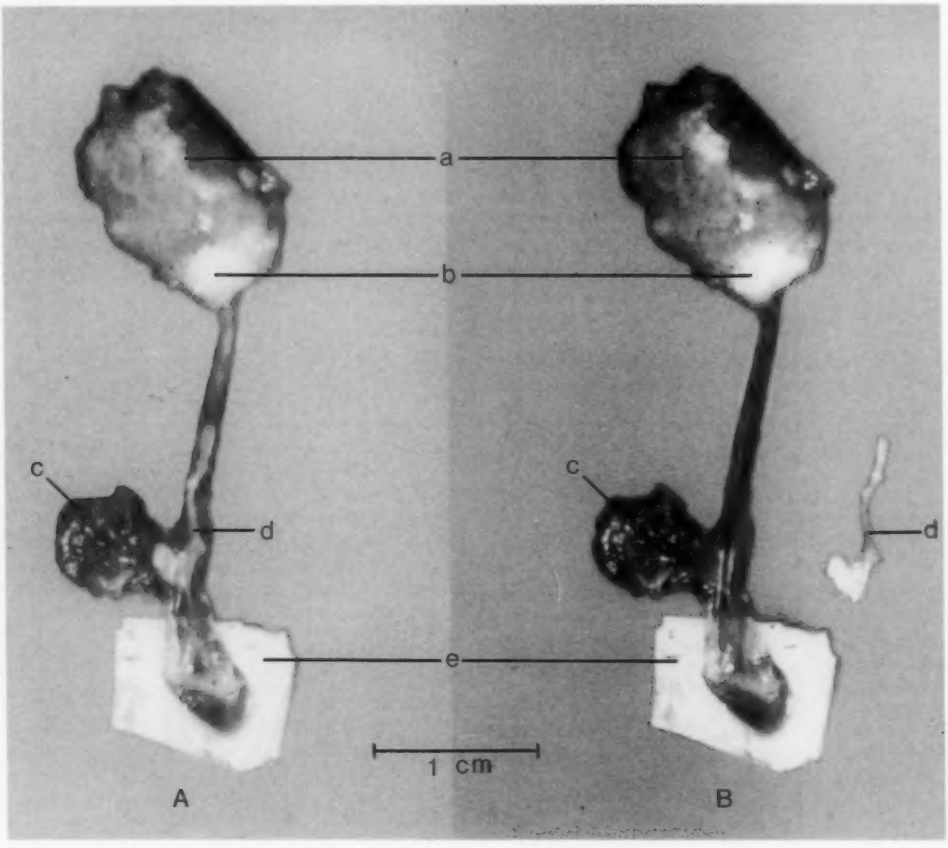
Fig. 1. Left reproductive tract dissected from an adult female Dungeness crab, 152 mm CW, collected off Eureka, California, 18 August 1977. The proximal sperm plug (A, *d*) is a complete clublike structure that is visible through the transparent vaginal wall and partially extends into a lateral evagination (bursa) off the vagina (see Jensen et al. 1996). In situ, ovaries are orange to dark red at this stage and are clearly distinguished from whitish material (sperm and seminal fluid) present in spermathecae. Initially, after mating, the sperm plug adheres to the vaginal wall and cannot be removed easily without destroying the vagina. After an unknown period of time following molting and mating, the sperm plug may be easily removed as a complete and discrete structure (B, *d*). Eventually, the sperm plug distintegrates or possibly may be pushed out when eggs are extruded. *a*, section of ovary; *b*, spermatheca; *c*, bursa; *d*, sperm plug; *e*, section of exoskeleton around vulva. Photo by P.W. Wild.



fecundity data previously reported by Hankin et al. (1989) for comparison with data collected in 1992 and 1993.

Calculation of egg production by carapace width interval

From the 1992 regression of log fecundity against log CW (for crabs in categories 1 and 2; see Results), fecundity was predicted from postmolt CW for the 411 female crabs measured in premating embraces during 1992. Postmolt CW was estimated for 233 of these crabs on the basis of a least-squares linear regression of postmolt CW against premolt CW for the sample of 178 female crabs that molted to known postmolt CW in the laboratory during 1992. Predicted egg production of these female crabs was summed over 5-mm postmolt CW intervals.

Results

Carapace width frequencies of adult female Dungeness crabs

CW frequency histograms for adult female Dungeness crabs collected near Trinidad, California, at depths of 14–28 m during mid-June and mid-July of 1992 through 1995 were similar; in each year, more than two thirds of all crabs were in the range of 130–155 mm CW (Fig. 2). Including comparable data collected in 2 earlier years (1982 and 1983; Diamond 1983), percentages of female crabs exceeding 150.0 mm CW ranged from 13.8 to 24.2% and averaged 17.4%; percentages of female crabs in excess of 155.0 mm CW ranged from 4.0 to 9.7% and averaged 6.4% (Table 1).

Smaller adult female Dungeness crabs were represented to a larger extent in CW frequency data collected by T.H. Butler during 1969 and 1970 (Fig. 3). As a consequence, percentages of large female crabs were lower than for the northern California data. For Butler's data, collected at depths of 5–11 m using closed-port commercial crab traps, percentages of female crabs exceeding 150.0 mm CW ranged from 1.7 to 3.4% and averaged 2.5%; percentages of females crabs in excess of 155.0 mm CW ranged from 0.2 to 1.0% and averaged 0.6% (Table 2).

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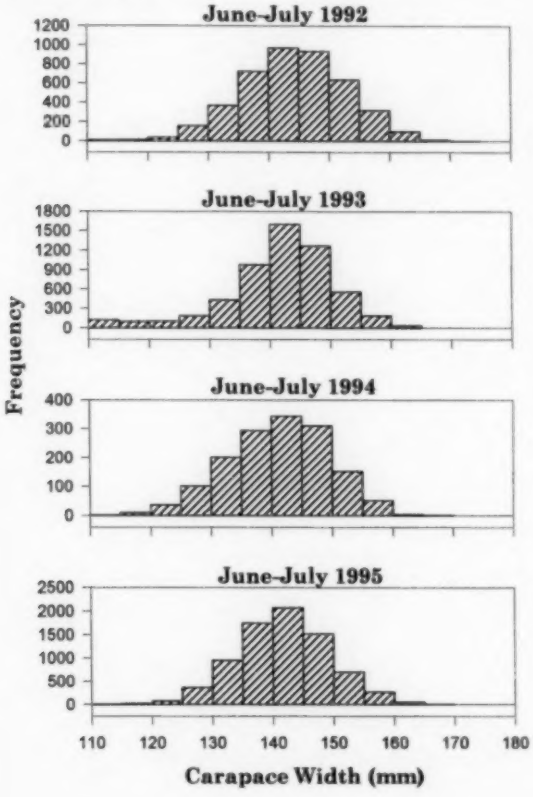
Fig. 2. Carapace widths of adult female Dungeness crabs collected off northern California during June and July of 1992–1995 using experimental traps nonselective with respect to female carapace widths exceeding 85 mm.



Delay from collection to molting of premating embrace females

The delay between collection of premating embrace females and their subsequent laboratory molting was known for 178 and 83 crabs collected in 1992 and 1993, respectively. The mean numbers of days from collection to molting were 1.66 and 3.64 days, respectively, in 1992 and 1993. The percentages of crabs that molted within 5 days of collection were 98.3 and 75.9%, and the maximum numbers of days until molting were 8 and 9 days, respectively, in 1992 and 1993.

Carapace widths of male and female crabs in premating embraces

Among premating embrace pairs of Dungeness crabs collected during 1992, 1993, and 1995 in northern California, premolt CW of females ranged from 80.9 to 159.9 mm, whereas CW of hard-shelled males ranged from 119.5 to 200.9 mm (Table 3). In all 3 years, substantial numbers of sublegal-sized males (<159 mm CW) were recorded in premating embraces (Fig. 4). The linear correlation between male CW and female premolt CW was weak and nonsignificant in 1993 ($r = 0.11$, $n = 101$), but similar weak correlations would be judged statistically significant in 1992 ($r = 0.18$, $n = 411$) and 1995 ($r = 0.14$, $n = 214$) when sample sizes were larger. The percentage of variation in male CW that could be explained by female premolt CW was extremely small in all years ($R^2 = 0.03$, 0.01, and 0.02, for 1992, 1993, and 1995, respectively).

Table 1. Numbers and percentages of adult female Dungeness crabs exceeding 150 and 155 mm CW among all adult female Dungeness crabs in excess of 110 mm CW captured in nonselective traps near Trinidad, California, during January–March 1982 and 1983 (approximated from Diamond 1983, Fig. 7), and during June–July 1992, 1993, 1994, and 1995 (this study).

Year	Total sample	No. >150 mm	No. >155 mm	% >150 mm	% >155 mm
1982	2682	527	211	20.0	8.0
1983	1182	256	117	21.7	9.5
1992	4369	1057	422	24.2	9.7
1993	6626	776	222	11.7	3.4
1994	1503	207	56	13.8	3.7
1995	7789	1022	317	13.1	4.1
Mean				17.4	6.4

Note: Data from 1982 and 1983 were collected from traps set off Clam Beach, California, at approximately 22 m; data for 1992–1995 were collected from traps set off Clam Beach and Big Lagoon, California, at depths of approximately 14–28 m.

Squared correlations for linear regressions of postmolt female CW against premolt CW were large ($R^2 = 0.97$, 0.97, and 0.88, for 1992, 1993, and 1995, respectively; Table 3). Thus, when necessary, postmolt female CW could be predicted with good accuracy from premolt CW (Fig. 5). When male CW was plotted against observed or predicted female postmolt CW, it became apparent that the minimum CW of male crabs almost always exceeded postmolt CW of females (Fig. 6). The maximum CW of males collected from premating embraces seemed to be unrelated to female postmolt CW, suggesting that large males are as likely to pair with small females as with large females, but there was a notable decrease in the range (and variation) of male CW with increasing female postmolt CW for the large 1992 data set. For the 1992 data set, this decrease in the range of male sizes reflected an apparent increase in minimum male size with increasing female postmolt size. There were several instances where male CW was a few millimetres less than female postmolt CW, however, and there was no indication that minimum male size must exceed female size by any substantial amount (as might have been inferred from plots of male CW against female premolt CW; Fig. 4).

Male CW was weakly but significantly correlated with female postmolt CW in 1992 ($r = 0.18$, $n = 411$) and 1995 ($r = 0.14$, $n = 214$), but not in 1993 ($r = 0.12$, $n = 101$). No more than 3% of the variation in male CW could be explained by linear regression on female postmolt CW in any year.

Premating embrace data collected by T.H. Butler in British Columbia during the 1950s were largely consistent with northern California data from 1992 to 1995. Substantial numbers of sublegal-sized male Dungeness crabs (<154 mm CW) were observed in premating embraces, and male CW almost always exceeded predicted female postmolt CW (Fig. 7). Again, however, there was no indication that minimum male CW must exceed female postmolt CW by any substantial amount. Male CW was not significantly correlated with female premolt CW ($r = 0.14$, $P = 0.17$, $n = 92$) or with female postmolt CW ($r = 0.14$, $P = 0.17$, $n = 92$).

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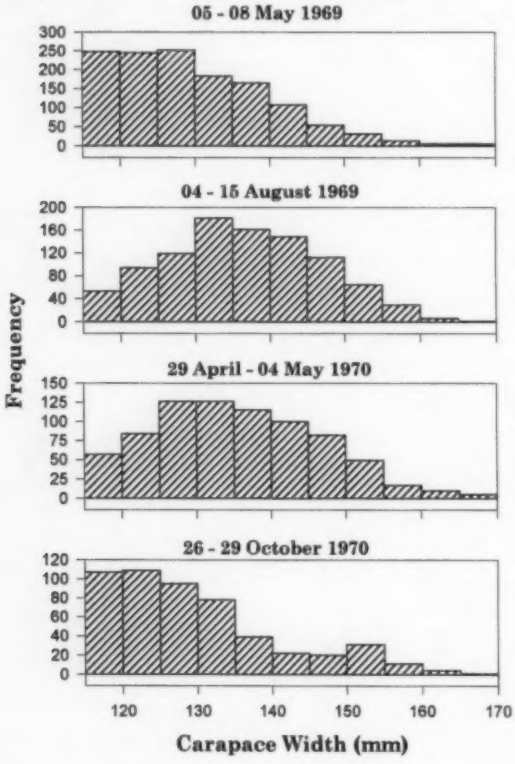
Fig. 3. Carapace widths (including spines) of adult female Dungeness crabs collected by Butler on Roberts Bank off the mouth of the Fraser River, British Columbia, during 1969 and 1970. Carapace width measurements excluding spines are approximately 0.95 times measurements including spines. Thus, the carapace width range of 120–170 mm including spines corresponds to a range of approximately 114–161.5 mm excluding spines.



Mean CW of premating embrace females increased over the course of the mating seasons in 1992, 1993, and 1995 (Fig. 8), thus implying that larger female crabs usually molted later than smaller female crabs. Fitted linear regressions of mean female premolt CW against 10-day calendar date of collection were highly significant ($P < 0.01$) in each year.

Presence of sperm plugs and sperm

In the sample of 587 female Dungeness crabs examined for presence of sperm plugs and for presence of sperm in spermathecae, 248 were classified as having definitely molted; 137, as having probably molted; 130, as having probably not molted; and 72, as having definitely not molted. The mean durometer measurement for those crabs classified as having definitely molted was significantly lower (52.9 durometer units; t test, $P < 0.001$) than means for other molting history categories (63.1–64.9 durometer units; Table 4).

Complete proximal sperm plugs (see Jensen et al. 1996) were present in 69% of all female crabs classified as having definitely molted but were essentially absent (<3%) in all other molting history categories. Sperm presence in spermathecae was categorized as swollen and full in 71% of female crabs

Table 2. Numbers and percentages of adult female Dungeness crabs exceeding 150 and 155 mm CW among all adult female Dungeness crabs in excess of 110 mm CW captured by T.H. Butler in closed-port commercial traps, 1969–1970.

Year	Sampling dates	Total sample	No. > 150 mm	No. > 155 mm	% > 150 mm	% > 155 mm
1969	5–8 May	1308	22	8	1.7	0.6
	4–15 August	988	25	4	2.5	0.4
1970	29 April – 4 May	771	26	8	3.4	1.0
	26–29 October	517	12	1	2.3	0.2
Mean					2.5	0.6

Note: Traps were set at 5–11 m depth on Roberts Bank near the mouth of the Fraser River, British Columbia.

classified as having definitely molted but in only 19–32% of other molting history categories. Absence of sperm in spermathecae was noted only for a few crabs classified as probably or definitely not having molted (Table 4).

Among those 248 female crabs classified as having definitely molted, 40 crabs were at least 150 mm CW. Sperm presence in spermathecae of these large female Dungeness crabs was classified as swollen and full in 29 of 40 crabs (72.5%) as compared with 147 of 208 (70.7%) smaller crabs. Although there was no obvious difference in sperm presence in spermathecae among definitely molted crabs of different CW, sperm plugs were absent in only 1 out of 40 (2.5%) crabs at least 150 mm CW as compared with 43 of 208 (20.7%) smaller molted crabs (Table 5).

Fecundities

Carapace widths for ovigerous females ranged from 116.4 to 162.7 mm in 1992 and from 123.6 to 165.8 mm in 1993. Estimated fecundities ranged from approximately 300 000 to 1 500 000 eggs in 1992 and from approximately 100 000 to 1 300 000 eggs in 1993 (Table 6). Of the 100 fecundity estimates generated for 1992, 30 were predicted from a fitted regression of 70 subsample estimates of fecundity (F) against the weight of the dried egg mass (W): $F = 44\,053 + 54\,202\,W$. This regression accounted for about 93% of the variation in estimated fecundities.

When the full model (eq. 1) was fitted to log fecundity, log CW, and molting history data for 1984, 1992, and 1993, significant effects of molting history on slope and elevation were detected for 1984, and a significant effect of molting history on elevation was detected for 1992 and for 1993. For 1984 data, log fecundity increased with log CW among molted crabs, but size-specific fecundities actually decreased with increasing log CW among nonmolted crabs (Table 7). When parameter estimates were transformed back to original units of measurement, the fitted fecundity (F) – carapace width (L) relation for molted crabs in 1984 was $F = 1.9682L^{2.5907}$. For 1992, estimated fecundity – carapace width relations were $F = 2884L^{1.1709}$ for molted crabs (shell condition categories 1 and 2) and $F = 1915L^{1.1709}$ for nonmolted crabs (shell condition categories 3 and 4). For 1993, estimated fecundity – carapace width relations were $F = 2.9472L^{2.5144}$ for molted crabs (shell condition categories 1 and 2) and $F = 1.9744L^{2.5144}$ for nonmolted crabs (shell condition categories 3 and 4). Thus, for a given CW, expected fecundities of nonmolted crabs were approximately 66.4 and 66.9% of fecundities of crabs that had

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Table 3. Summary of premating embrace data and postmolt–premolt carapace width regression statistics for data collected during 1992, 1993, and 1995 off Trinidad, California.

	Summarized premating embrace data					Postmolt–premolt OLS regression statistics		
Year	Female size range (mm)	Male size range (mm)	No. of pairs	No. of known postmolt sizes	Total no. of molt increments	Intercept	Slope	R^2
1992	101.8–159.9	119.5–199.1	411	157	178	29.56	0.8822	0.9725
1993	80.9–137.7	137.0–200.9	101	83	83	34.69	0.8361	0.9770
1995	108.4–142.0	130.0–186.8	215	0	155	22.54	0.9399	0.8810

Note: Known postmolt sizes refers to laboratory-generated molt increments for females removed form mating embraces for which male size was known. OLS, ordinary least-squares.

Fig. 4. Male carapace widths plotted against female premolt carapace widths for Dungeness crabs collected in premating embraces in northern California near Trinidad during 1992, 1993, and 1995. The horizontal lines at 159 mm indicate the minimum CW of legal-sized male crabs in California, Oregon, and Washington fisheries.

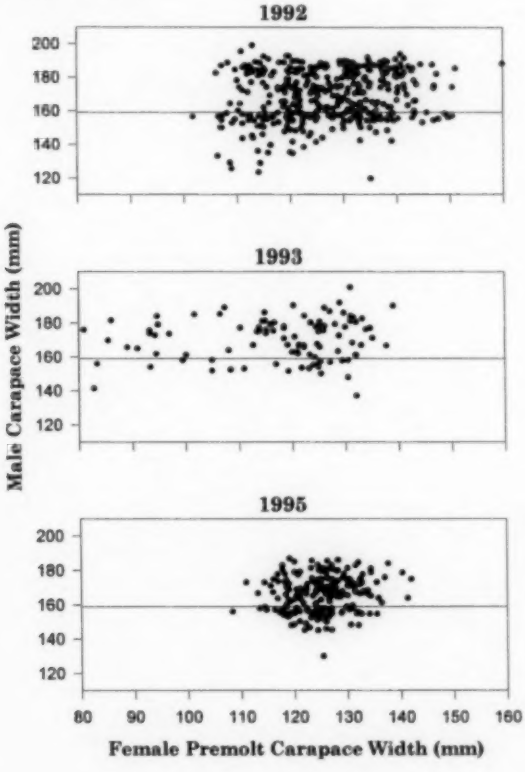
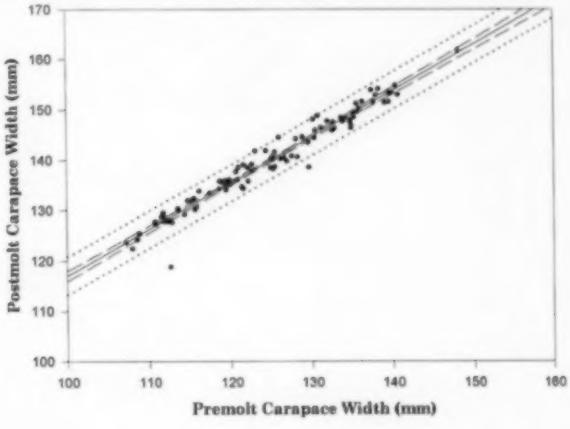


Fig. 5. Postmolt carapace width plotted against premolt carapace width for 178 female Dungeness crabs removed from premating embraces in northern California during 1992. Solid line is the fitted regression line; dashed lines give 95% confidence intervals for expected postmolt CW given premolt CW; dotted lines give 95% confidence interval for predicted postmolt CW given premolt CW.



molted (and presumably mated) in 1992 and 1993, respectively.

Although significant effects of molting history were detected for all three fecundity samples, most of the variation in size-specific log fecundities remained unexplained by regression on log CW and molting history; regressions only explained 16–41% of observed variation (Table 7). Interannual variation in expected size-specific fecundities among molted female Dungeness crabs was also relatively large, especially among smaller females. For example, expected fecundities during 1984, 1992, and 1993 were about 479 000, 784 000, and 498 000 at CW = 120 mm; about 715 000, 939 000, and 734 000 at CW = 140 mm; and about 1 010 000, 1 098 000, and 1 027 000 at CW = 160 mm. Nevertheless, the qualitative patterns of size-specific fecundities for molted compared with nonmolted crabs were visually similar in all years (Fig. 9).

Egg production by carapace width interval

The frequency histogram (total $n = 411$) of observed ($n = 178$) and predicted ($n = 233$) postmolt CW for female Dungeness crabs collected from premating embraces during 1992 was very similar to the frequency histogram constructed for all crabs measured during June and July 1992 (Fig. 10). The good qualitative agreement between these two histograms is in part a reflection of the fact that almost all adult female Dungeness crabs were classified as having molted (classes 1 and 2) during 1992, irrespective of CW (Hankin and Xue 1996). Of the 411 female Dungeness crabs collected in premating embraces during 1992, 83 crabs (20.2%), 26 crabs (6.3%), and 7 crabs (1.7%) had observed or predicted postmolt CW in excess of 150, 155, and 159 mm, respectively. For this same sample of 411 female crabs found in premating embraces, the percentages of total estimated egg production accounted for by crabs

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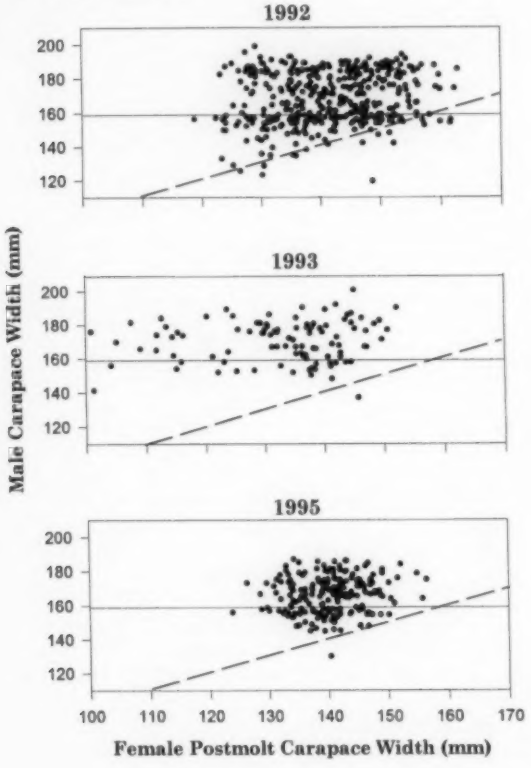
Fig. 6. Male carapace widths plotted against observed and (or) predicted female postmolt carapace widths for Dungeness crabs collected from premating embraces in northern California near Trinidad during 1992, 1993, and 1995. Horizontal lines indicate the minimum CW (159 mm) of legal-sized male crabs in California, Oregon, and Washington fisheries. Broken lines are lines along which CW of male crabs exactly equals CW of female crabs. In northern California, the minimum postmolt CW for ovigerous females is about 110 mm.



with CW in excess of 150, 155, and 159 mm were 24.8, 7.6, and 2.1%, respectively. (Fig. 11).

Discussion

Because the minimum CW of mating male crabs generally exceeds the postmolt CW of female crabs, it is reasonable to conjecture that intensive fishing, through reduction of the abundance of large males, might reduce mating success among large female crabs, thereby reducing egg production by a female stock of Dungeness crabs (Smith and Jamieson 1991). Although such conjectured effects are reasonable, the actual realized effects of fishing on mating success must depend on relations among at least the following factors: (*i*) minimum size limits for male crabs; (*ii*) magnitude of fishery exploitation rates; (*iii*) population size structure and molting patterns of female crabs as they compare with (*i*) and (*ii*); (*iv*) size requirements and (or) preferences of males and females for mating as they compare with (*i*), (*ii*), and (*iii*); and (*v*) durations of mating activities of individual pairs as compared with the

Fig. 7. Male carapace widths plotted against female premolt carapace widths (A) and against female postmolt carapace widths (B) for 92 pairs of Dungeness crabs collected by T.H. Butler in premating embraces at Queen Charlotte Islands, British Columbia, July 1951 through July 1957. Postmolt carapace widths (Y) were predicted from premolt carapace widths (X) on the basis of linear regression ($Y = 28.50 + 0.91X$, $R^2 = 0.98$). Horizontal lines indicate the minimum CW (154 mm) of legal-sized male crabs in British Columbia fisheries. The broken line is a line along which CW of male crabs exactly equals CW of female crabs.

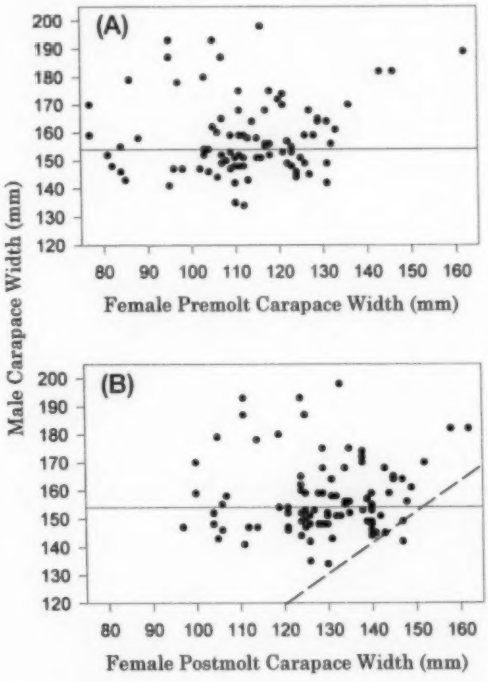


Fig. 8. Mean premolt carapace width (symbols) by 10-day periods plotted against the lower limit of the period (calendar date) for female Dungeness crabs found in premating embraces off northern California in 1992, 1993, and 1995. Broken lines display fitted linear regressions of means against calendar date. Error bars are ±1SE.

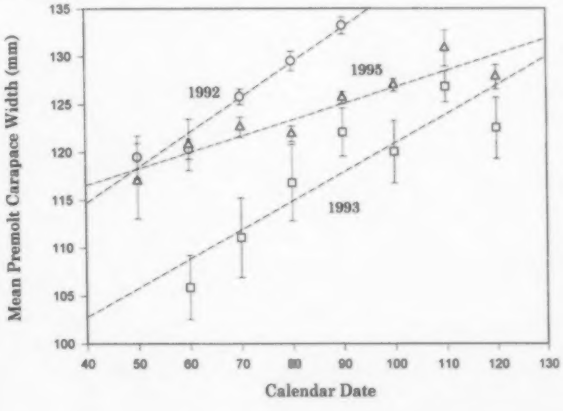


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Table 4. Shell condition classification, sample size, shell hardness measured in durometer units (mean ± SD), sperm plug presence (1, complete plug visible; 4, no plug visible), sperm presence in spermathecae (1, swollen and full; 4, none present), and carapace widths (range and mean) for female Dungeness crab dissected shortly after completion of the spring molting season, June 1995.

Shell condition classification	Sample size	Shell hardness	Sperm plug presence				Sperm presence in spermathecae				Carapace width (mm)	
			1	2	3	4	1	2	3	4	Range	Mean
Definitely molted (class 1)	248	52.9±8.99	172	26	6	44	176	55	17	0	113.2–162.0	141.9
Probably molted (class 2)	137	63.1±4.46	3	5	10	119	44	63	30	0	120.6–161.4	138.0
Probably not molted (class 3)	130	64.8±4.39	1	2	3	124	29	69	30	2	125.7–162.0	142.8
Definitely not molted (class 4)	72	64.9±6.02	2	2	2	66	14	40	17	1	132.8–165.2	147.2

Table 5. Carapace widths (range), sample size, sperm plug presence (1, complete distal part of sperm plug visible; 4, no plug visible), and sperm presence in spermathecae (1, swollen and full; 4, none present) for female Dungeness crabs classified as having definitely molted on the basis of dissections made shortly after completion of the spring molting season, June 1995.

Carapace width	Sample size	Sperm plug presence				Sperm presence in spermathecae			
		1	2	3	4	1	2	3	4
150.0–162.0	40	32	5	2	1	29	8	3	0
140.0 –149.9	106	83	8	1	14	83	21	2	0
130.0–139.9	87	53	8	3	23	55	23	9	0
120.0–129.9	13	3	4	0	6	7	3	3	0
110.0–119.9	2	1	1	0	0	2	0	0	0

durations of the female molting seasons in given populations. The minimum size limit and fishery exploitation rate together determine the fraction of legal-sized males that are available for mating; the size requirements and (or) mating preferences of males and females together with the population size structure and molting patterns of females determine the degree to which available unharvested males are large enough for mating; and the duration of a pair's mating activities relative to the female molting season determines the degree to which individual males may be polygynous. These five factors should also together determine the major impacts of fishing on population-level egg production through their direct effects, if any, on mating success of females. We distinguish such potential first-order effects on egg production from more modest second-order effects on egg production that may result from (*vi*) dependence of fertilization success on sperm viability or volume as a function of male size.

In this paper, we addressed the issue of mating success using two complementary approaches. Our first approach was to collect extensive information regarding factors *iii*, *iv*, and *v*, above. We examined the implications of these data given the existing minimum size limit (159 mm CW) and high exploitation rates (often up to 90% or more) in the northern California population of Dungeness crabs. We also examined the implications of similar though less extensive data collected by T.H. Butler in British Columbia during the 1950s and 1970s given the British Columbia size limit of 154 mm CW. Such information and analysis can allow one to make reasonable inferences or hypotheses regarding the potential effects of fishing on mating success, but these hypotheses would remain untested in the absence of direct evidence of actual mating success. Our second approach was therefore to collect direct empirical evidence of mating success. We accomplished this through dissection and examination of the reproductive tracts of a large sample (n = 587) of adult female Dungeness crabs with a wide range of size and molting history. This latter approach is similar, in principle, to that used by Wenner (1989) in her assessment of mating success among female blue crabs, *Callinectes sapidus*. Wenner's (1989) research was motivated by similar concerns regarding possible reduction in mating success of female blue crabs resulting from intense removal of male blue crabs.

Our direct empirical assessments of mating success in northern California provided no evidence in support of a contention that larger molted female Dungeness crabs receive less attention than smaller molting females. Indeed, the percentage of large (>150 mm CW) molted females that showed physical evidence of mating (presence of sperm plugs) in spring 1995 was exceptionally high (97.5%, 39 of 40) and was higher than analogous percentages for molted females of smaller size categories; percentages were 89.3% (92 of 103) and 73.6% (64 of 87) for molted female crabs in size intervals 140.0–149.9 and 130.0–139.9 mm, respectively.

We believe that it would be incorrect to interpret these lower percentages of sperm plugs as indicating that smaller female Dungeness crabs receive less attention than larger female crabs. First, there were clear differences in sperm presence ratings between crabs that were classified as having definitely molted (71% rated as swollen and full, irrespective of size) and crabs that had probably or definitely not molted (21% ranked as swollen and full). Because large females (>150 mm CW) comprised only 17% of all molted females, it is apparent that most smaller females must have mated during spring 1995. Second, our ratings of sperm presence in spermathecae, in contrast to our ratings of sperm plug presence, showed no tendency for smaller molted female crabs to have lower sperm presence ratings than large molted female crabs (Table 5). Thus, our sperm presence data are consistent with a conclusion that molted females of 130–150 mm CW probably had mating success similar to that of larger females. We instead attribute the differences in percentages of observable sperm plugs and (or) remnants of sperm plugs to (*i*) the tendency of smaller female Dungeness crabs to molt and mate earlier in the molting season than larger female crabs (Fig. 8), and to (*ii*) disintegration of the sperm plug.

The tendency of smaller adult female crabs to molt and mate earlier in a population's overall molting season than larger adult female crabs has also been observed in the closely related species *Cancer gracilis* (Orensanz et al. 1995; J.M. Orensanz, University of Washington, Seattle, WA 98195, personal communication). Lipcius (1985) proposed that this tendency is a general phenomenon among long-lived decapods.

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Table 6. Summary of fecundity data for female Dungeness crabs collected in 1984, 1992 (see text for category definitions), and 1993 off northern California.

Year	Sample size	Carapace width range (mm)	Range of fecundity estimates	Shell condition				
				1	2	2–3	3	4
1984	89	110.3–167.4	122 336 – 1 545 940	17		55		17
1992	100	116.4–162.7	301 254 – 1 519 620	47	25	13	6	9
1993	66	123.6–165.8	94 391 – 1 288 843	20	15	13	16	2

Note: Values for shell condition are numbers of crabs within each shell condition classification category.

Table 7. Summary of results of fitting dummy variable multiple regression models of the relation between log fecundity, log carapace width, and molting history (molted and probably molted, categories 1 and 2, contrasted with not molted and probably not molted, categories 3 and 4) for ovigerous female Dungeness crabs collected from commercial crab traps during January–February of 1992 (1992) and for crabs collected while nonovigerous during August–October of 1992 but later extruding egg masses in the laboratory (1993).

Year	Sample sizes			Regression model parameter estimates				Regression statistics			
	Total crabs	Molted crabs	Not molted crabs	B_0	B_1	D_0	D_1	R^2	F	df	P
1984	34	17	17	0.6771	2.5907	8.4435	41.9389	0.4096	6.9470	3,30	<0.01
1992	87	72	15	7.9669	1.1709	0.3930		0.3051	18.4426	2,84	<0.00001
1993	53	35	18	1.0809	2.5144	0.4006		0.1630	4.8750	2,50	0.0110

Although it does not appear clear whether the sperm plug is produced by the male (Orensanz et al. 1995) or sperm plug formation is induced by penetration of male sperm and seminal fluid into the vagina of females (Diesel 1991), it does seem clear that sperm plug presence is a definitive indicator of mating activity in Dungeness crabs (Jensen et al. 1996). Jensen et al. (1996) did not follow the fate of sperm plugs through to complete disappearance (experiments were limited to a 20-day period following mating). In our own research on Dungeness crabs, substantial evidence of sperm plugs was found only among crabs classified as having definitely molted; evidence of sperm plugs was essentially absent among crabs from all other molting categories, including the category classified as probably having molted. It is our belief that those crabs that we classified as probably having molted are those that molted early during spring 1995; often these were very small adult female crabs (110–125 mm postmolt CW). Presumably, the delay from molting and mating to collection and examination of such females was sufficiently long that deterioration of the sperm plug was essentially complete. We intend to examine sperm plug deterioration in detail in the near future.

The function of the sperm plug in Dungeness crabs is not certain. In general, sperm plugs may serve to prevent sperm competition in the event that more than one male crab were to mate with a single female crab (Diesel 1991; Orensanz et al. 1995). Laboratory matings have confirmed that female Dungeness crabs can be mated by more than one male (Jensen et al. 1996; D.G Hankin, unpublished observations). The possible role of the sperm plug in preventing sperm competition in Dungeness crabs, however, is complicated by the presence of the unusual sac (or bursa) in the female reproductive tract (see Fig. 1; Jensen et al. 1996). According to Jensen et al. (1996), sperm from a second male may be deposited and stored in this bursa even if a sperm plug has formed from a previous mating. The function of sperm stored in the bursa is currently unknown.

Although sperm presence received lower ratings among crabs classified as having probably or definitely not molted than among those classified as having definitely molted, sperm presence was rated as absent in only 3 of all 587 females, irrespective of molting history. Retention of sperm by nonmolted or skip-molt females is consistent with earlier reports that nonmolting female Dungeness crabs can retain sperm for at least 2.5 years and then produce viable egg masses (Hankin et al. 1989); with reports of recoveries of ovigerous tagged female crabs that had been at large through more than two molting seasons but had not changed size (Pacific Fisheries Management Council 1979, p. 65); with laboratory reports of four individual females extruding viable egg masses over 2 successive years without mating, two of which also molted (Wild et al. 1978); and with Shirley and McNutt's (1989) finding that small female Dungeness crabs (80–117 mm CW) can mate, "molt during the next year, and without subsequent mating, extrude eggs and incubate normally developing embryos." Although female Dungeness crabs can rely on stored sperm for fertilization of successive egg masses in the absence of molting and mating, we have provided additional evidence confirming Hankin et al.'s (1989) earlier conclusion that size-specific fecundities among nonmolting female Dungeness crabs are generally reduced when compared with size-specific fecundities of molted crabs (Table 7, Fig. 9). Although reduced sperm presence (volume) or viability may limit fertilization success and fecundity among nonmolting females, it is also possible that reduced fecundity reflects reduction in attachment surface owing to continuing physical damage to pleopods. Pleopod damage goes unrepaired among crabs that extrude eggs without molting, and it is common to observe such damage among nonmolted female Dungeness crabs in northern California.

Our direct empirical assessment of mating success was made from a single, albeit large, sample collected in a single year (1995) at a single location (off Trinidad, Calif.). For a variety of reasons, it might be unreasonable to conclude that the apparent near 100% mating success among large females that we observed in 1995 was representative of mating success of large female Dungeness crabs in most years. We therefore now consider the implications of information, collected over

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Fig. 9. Log estimated fecundity plotted against log carapace width for female Dungeness crabs collected in northern California during 1984, 1992, and 1993. Open circles are for crabs classified as definitely having molted or probably having molted (categories 1 and 2) during the most recent molting season; solid circles are for crabs classified as probably not molted or definitely not molted (categories 3 and 4). Samples sizes (all categories combined) were 34, 87, and 53 for 1984, 1992, and 1993, respectively.

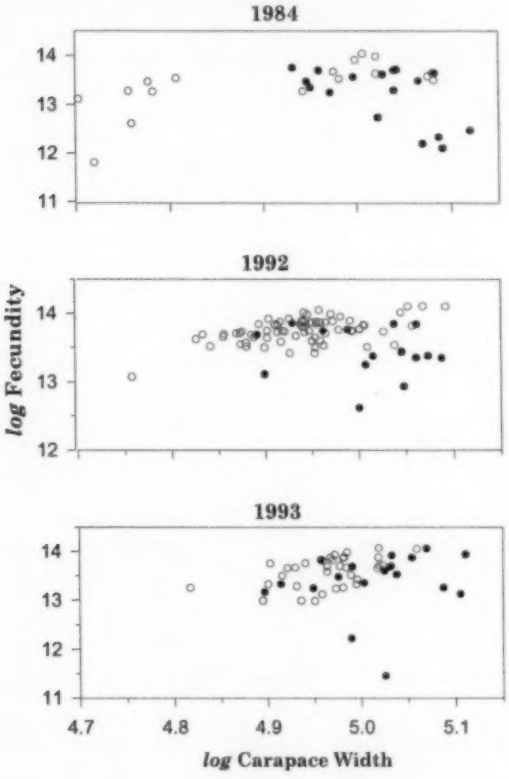
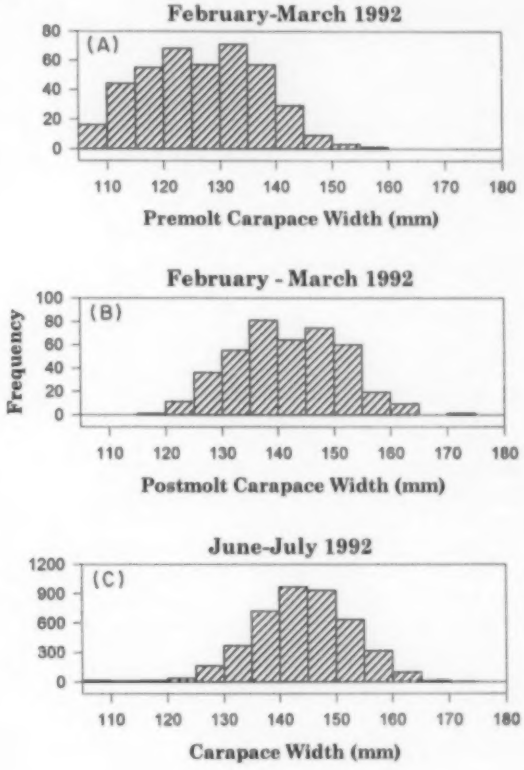


Fig. 10. Frequency histograms for measurements of premolt (A) and postmolt (B) carapace widths of 411 female Dungeness crabs collected in premating embraces in northern California during 1992, as contrasted with frequency histogram for all adult female Dungeness crabs collected using nonselective experimental traps during June and July of 1992 (C). If size frequencies of female Dungeness crabs collected in premating embraces were representative of the size structure of the entire population of adult crabs engaged in mating activity then, if all crabs molted, the distribution of postmolt carapace widths in B would be similar to that in C.



a greater number of years, concerning those factors that must together affect mating success.

The extensive CW frequency data that we present in this paper leave little doubt in our minds that female crabs exceeding the minimum CW of legal male crabs comprise a very small fraction of the total populations of adult female Dungeness crabs that we sampled in northern California and British Columbia. In our most recent northern California samples, proportions of adult female Dungeness crabs exceeding legal size for males (159 mm) were 3.5, 0.8, 0.5, and 1.8% for 1992–1995, respectively; comparable sample percentages for 1982–1983 were approximately 2–4%. In Butler's 1969 and 1970 British Columbia collections from closed-port commercial traps, less than 1% of sampled crabs exceeded minimum legal size for males (154 mm CW). Butler's British Columbia data were collected at shallower depths (5–11 m) than northern California data (14–28 m) and may overly represent smaller and possibly immature female crabs. Alternatively, Butler may have encountered some abnormally large year-classes that dominated catches of small crabs in 1969 and 1970. In any event, Butler used closed-port commercial crab traps that were size selective (i.e., allowed some small adult female crabs to escape; see Diamond 1983; Diamond and Hankin 1985) when compared with the nonselective experimental traps used in this study. We have not adjusted Butler's data for such gear selection because this would only have further reduced the calculated proportions of large female crabs among these British Columbia samples.

One may, of course, legitimately question the correspondence between size frequencies of crabs collected from traps and size structure in the true population. In particular, size structure and (or) molting histories of female Dungeness crabs may vary substantially according to depth, month, and location. Although our sampling was relatively limited in scale, we attempted to address such variation by collecting samples at two depths, two months, and two locations in each year. As a working hypothesis, we believe that it is reasonable to assume that our size-frequency data provide useful approximate descriptions of true population size structure.

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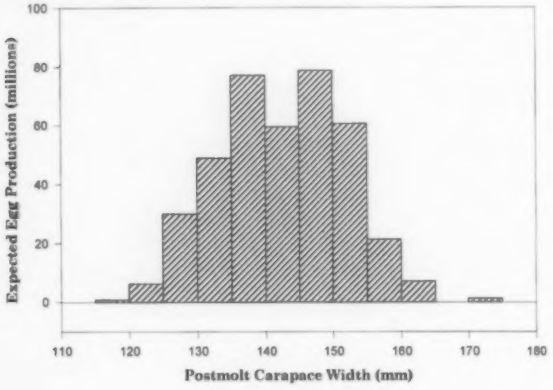
Fig. 11. Expected egg production plotted against postmolt CW for 411 female Dungeness crabs removed from premating embraces in northern California during 1992. Expected size-specific fecundities were predicted from a fitted linear regression of log fecundity against log postmolt CW in a sample of 100 ovigerous crabs for which fecundities were estimated during 1992.



It is important to recognize that our northern California size-frequency data were collected following the spring molting and mating seasons for females. The size frequencies that we observed thus reflected postmolt sizes for those females that molted in a given year. In our analysis of sizes of male and female Dungeness crabs collected from premating embraces, we plotted male CW against both female premolt CW (Fig. 4) and observed or predicted female postmolt CW (Fig. 6). From the plots of male CW against female postmolt CW, we concluded that mating male Dungeness crabs must have a minimum CW that is approximately the same as female postmolt CW. In northern California, it is thus logical to conclude that sublegal male Dungeness crabs with CW less than 159 mm would be suitable mates for female Dungeness crabs with postmolt CW less than, say, 155 mm. As the percentage of female Dungeness crabs with postmolt CW exceeding 155 mm is generally very small (3–10% of adult females; Table 1) in northern California, it is reasonable to conclude, as a first approximation, that a shortage of legal-sized males would restrict mating success in only a very small percentage of the female population. Similar arguments would apply to the Fraser River British Columbia data collected by T.H. Butler (Table 2, Fig. 3), although the minimum size limit for male crabs is 154 mm in British Columbia. Northern California and British Columbia premating embrace data provide substantial evidence that sublegal-sized males successfully participate in mating, confirming an essential underlying precept of existing minimum size limits for male crabs. The successful participation of sublegal-sized males in mating activities is also supported by British Columbia male mating mark data collected by T.H. Butler in the 1950s (Butler 1960) and more recently by Smith and Jamieson (1991).

Our premating embrace data provided no indications that larger male Dungeness crabs prefer larger female crabs as mates; maximum male size in mating embraces appeared to be independent of female size. We believe that the weak (but statistically significant owing to large sample sizes) positive linear correlations that we calculated between male CW and female postmolt CW are due to the increase in minimum male CW with increasing female postmolt CW and not to any tendency for large males to select large females as mates. Orensanz et al. (1995) found no indications of assortive mating in *Cancer gracilis*. Reid et al. (1991) found no tendency of male shore crabs, *Carcinus maenas*, to preferentially choose larger mates, although there appears to be mild evidence of assortive mating in this species.

It does generally appear to be the case, however, that larger males are more successful in securing mates than are smaller males when they are placed in direct competition in artificial laboratory settings. For at least *Carcinus maenas* (Berrill and Arsenault 1982), *Cancer gracilis* (Orensanz et al. 1995), and *Cancer pagurus* (Edwards 1966), larger male crabs almost always win such competitions for female mates. Thus, although smaller males may often be suitable for mating, competitive behaviors among male crabs may generally favor greater mating success by larger males. This kind of size dominance of mating success among males must surely bias collected observations of male and female sizes in premating embraces. Hypothetically, in an unfished population of Dungeness crabs, one might expect to observe much less mating activity by sublegal-sized crabs because smaller males would be less successful when competing with an abundance of larger males.

Our conclusion, from existing premating embrace data and size-frequency data, that a relatively small percentage of adult females should be affected by fishery-induced elimination or severe reduction of males exceeding legal size limits does require clarification and elaboration in several important respects. First, it is conceivable that mating success is limited not by the sizes of available male crabs but instead by the numerical abundance of males of a suitable size. The above arguments implicitly assume that there is no numerical shortage of males below legal size and (or) that male crabs are polygynous. Second, premating embrace data, especially recent data from northern California, provide abundant evidence that large legal-sized males do somehow manage to actively participate in mating, despite intensive fishing. Third, the size-frequency data that we present in Figs. 2 and 3 include nonmolted or skip-molt females as well as those that molted. Because annual molting probabilities of female Dungeness crabs decline substantially with increasing premolt CW (Hankin et al. 1989; Hankin and Xue 1996), a substantial proportion of those females with measured CW in excess of, say, 155 mm are nonmolted crabs that rely on stored sperm from previous matings. Thus, the actual annual demand for mating among large females is exaggerated by the nominal size-frequency data that we have plotted.

As the second and third points noted above would only further reduce any concerns that one might have regarding mating success among large female Dungeness crabs, we elaborate further only on the first point. The degree to which male Dungeness crabs are polygynous must depend critically on the duration of mating activities of individual pairs as compared with the duration of the molting season for females. Our laboratory records of the delays between collection and molting of premating embrace females suggest that the period of premating embrace is short (mean delays of 1.66 and 3.64 days and maximum delays of 8 and 9 days in 1992 and 1993, respectively). If we assume an average trap soak time of 3 days prior to collection, we can obtain crude estimates of the duration

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of premating embrace behaviors: 5 and 7 days with upper limits of 11 and 12 days in 1992 and 1993, respectively. (*Cancer gracilis* exhibits a similar duration of premating embrace behaviors (Orensanz et al. 1995, their Fig. 9).) From Snow and Nielsen (1966) and our own laboratory observations (D.G. Hankin, unpublished observations), the postmating embrace lasts no more than 2 days. We thus estimate that the mean durations of mating activities for individual pairs were perhaps 7 and 9 days with upper limits of 13 and 14 days in 1992 and 1993, respectively. When compared with the approximately 12-week duration of the molting season in northern California, an average male Dungeness crab could conceivably mate with 6–12 female Dungeness crabs over the course of the molting season for females.

Our two complementary approaches to assessment of the effects of fishing on mating success lead us to complementary and compatible conclusions. Our direct empirical evidence of mating success, achieved via dissection of reproductive tracts of a large sample of postmolt females, supports a conclusion that virtually all large molting females mated during spring 1995 in the intensely harvested northern California population of Dungeness crabs. Our analysis of premating embrace data, size-frequency data, and recorded delays between collection and molting of female crabs collected from premating embraces suggests that only a very small percentage of the total adult female population of Dungeness crabs in northern California or in British Columbia might experience reduced mating success given existing minimum size limits for male crabs.

Potential effects of intense fisheries on total egg production of female Dungeness crabs cannot easily be assessed in any direct fashion, so we made hypothetical worst-case calculations producing plausible upper limits for such effects. For the sake of argument, we calculated expected total egg production for the sample of 411 female Dungeness crabs that were collected from premating embraces during 1992. The postmolt CW frequency histogram for these crabs was similar to that for all female Dungeness crabs collected in northern California in June and July of 1992 (Fig. 10). This good agreement between the two frequency histograms would be expected only if (*i*) premating embrace females were representative of all females engaged in molting and mating, and (*ii*) relatively few female Dungeness crabs did not molt in 1992. Indeed, Hankin and Xue (1996) noted that molting probabilities were unusually high in 1992; except for the very largest females, almost all adult females molted in that year. For the sake of argument, we assumed that no large female Dungeness crabs produced eggs; related calculations suggest that total egg production would be reduced by no more than 2–25% depending on numerical specification of minimum size for large female Dungeness crabs. These calculations ignore the variable additional contribution to total egg production that may arise from adult female Dungeness crabs that rely on stored sperm and extrude egg masses without molting and mating; in 1992, 1993, 1994, and 1995, percentages of nonmolted crabs among those crabs exceeding 150 mm CW were 5.1, 37.6, 16.9, and 21.6%, respectively.

Although our worst-case calculations of the potential effects on total egg production seem inconsiderable to us, there are other modest second-order effects on total egg production that might result from fishery removal of large males. Although there is substantial evidence that sublegal-sized male Dungeness crabs can and do serve as suitable mates for female Dungeness crabs, it is possible that larger male Dungeness crabs are superior mates: larger male crabs may deliver greater amounts of sperm to individual females or they may effectively mate with a greater number of females. Volume of sperm delivery could be important for relatively large female Dungeness crabs when they molt to a size beyond which further molting is unlikely. Subsequent egg production for such females would depend on long-term sperm storage without molting. Although an initial full clutch of eggs might be fertilized by the sperm provided by a sublegal-sized male Dungeness crab, subsequent clutches might not be fully fertilized if small males did not provide adequate volume or viability of sperm. This possibility is not inconsistent with observed reduced fecundity among nonmolted female Dungeness crabs. The effects on total egg production of Dungeness crabs of reduced participation of large males in mating would be small, however, because (*i*) the proportion of nonmolting crabs among the total adult female population is small and (*ii*) annual survival rates of large (>155 mm) typically nonmolted female Dungeness crabs are very low (about 10%; Hankin et al. 1989).

We are not aware of published research that has addressed the potential superiority of large males with respect to the above issues in *Cancer* crabs or in closely related species. Paul and Paul (1990*a*, 1992), Paul et al. (1995), and Sainte-Marie and Carriere (1995) have reported relevant but conflicting information of this type for the majid crabs *Chionecetes bairdi* and *Chionoecetes opilio*; these species are believed to exhibit terminal molts to maturity. In *Chionoecetes opilio*, reproduction appears more complex than in *Chionoecetes bairdi*, as marked differences between the sexes in size and age exist (Sainte-Marie and Carriere 1995). Paul and Paul (1990*b*) provided laboratory evidence for a size-dependent drop in fertilization success with successive matings among male king crab, *Paralithodes camtschaticus*. Because these studies have been carried out on species with quite different reproductive biology from *Cancer magister*, it is difficult to judge the extent to which they may apply to Dungeness crabs. Basic studies of the reproductive biology of Dungeness crabs, such as those recently carried out and (or) proposed by Jensen et al. (1996), should shed light on these topics in Dungeness crabs.

Finally, we contrast the nature of our conclusions with previously conjectured effects of fishing on mating success and egg production of female Dungeness crabs (Smith and Jamieson 1991). First, we have a strong difference of opinion regarding interpretation of male mating mark data. We regard the greater incidence of mating marks among sublegal-sized male crabs than among legal-sized male crabs (first noted by Butler (1960)) as providing additional strong empirical support for our conclusion that sublegal-sized males actively participate in mating. (Warner (1987) documents extensive mating marks on sublegal-sized male crabs in northern California.) Second, we believe that the apparent discrepancy between our interpretations of premating embrace data could be largely resolved if "CW" were replaced by "premolt CW" in their proposal that "females larger than 140 mm CW could have difficulty finding a mate if legal-sized males were rare" (Smith and Jamieson 1991). The expected postmolt CW of a female with premolt CW of 140 mm has ranged from 151 to 154 mm and has averaged about 153 mm in northern California (Hankin and Xue 1996).

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We remain in substantial disagreement with Smith and Jamieson, however, with regard to the extent to which reasonable inferences concerning the effects of fishing on mating success can be made in the absence of comparative data from intensely fished and unfished populations. Smith and Jamieson (1991) implied that the exceptionally large size of female Dungeness crabs collected from Holberg Inlet, British Columbia, must reflect "normal" female growth patterns in unfished populations, whereas smaller sizes observed elsewhere reflect fishery-induced shifts resulting from intensive harvest of male crabs. Although we agree with Smith and Jamieson (1991) that the exceptionally large size of Holberg Inlet female Dungeness crabs is interesting, we believe that their conclusion of fishery effects is seriously confounded by environmental and (or) genetic factors that may influence growth rates. If female Dungeness crabs from unfished populations regularly reached the large sizes (>190 mm CW!) of the Holberg Inlet population, we have no doubt that such sizes would have been reported in the early fisheries literature. We are not aware of such reports, and we cannot imagine a meaningful contemporary experiment that might mimic a virgin unexploited state for a Dungeness crab population. While we recognize that our own conclusions are to some unknown degree confounded by an absence of fished and unfished populations from which one might draw unambiguous conclusions regarding fishery effects, such a position is hardly a novel one for fishery scientists. One must do the best that one can to make sense out of the data that are available; we believe that we have made an honest attempt to do just that.

Acknowledgments

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Distribution and drift of Atlantic cod (*Gadus morhua*) eggs and larvae on the northeast Newfoundland Shelf

P. Pepin and J.A. Helbig

Abstract: We present the results of a study designed to ascertain the importance of variations in transport of cod eggs and larvae on the northeast Newfoundland Shelf. The distribution of cod eggs and larvae is described from a series of ichthyoplankton surveys. The mean and variance in surface currents were estimated from five experiments in which clusters of drogued satellite-tracked drifters were released at the presumed northern limit of the stock's range. These data were used to project the patterns of drift both backward and forward in time and thus the possible sources and destinations of eggs and larvae. Considering the development from the youngest (stage I) to the oldest stages (larvae), we saw a north-to-south and an offshore-to-inshore progression in spatial patterns of spawning and a continuing increase in the overall level of dispersion with increasing age of offspring. The data do not support the hypothesis that coastal or offshore areas represent simple sources or sinks of cod eggs and larvae. Transport plays an important but highly variable role that is coupled to the spawning distribution of adult fish and the availability of suitable conditions or habitats when the young begin to undergo metamorphosis and settle.

Résumé : Nous présentons les résultats d'une étude visant à établir l'importance des variations dans le transport des oeufs et des larves de morue sur la partie nord-est du plateau de Terre-Neuve. La distribution des oeufs et des larves est décrite à partir d'une série de relevés de l'ichtyoplancton. La moyenne et la variance des courants superficiels ont été estimées à partir de cinq expériences au cours desquelles des grappes de dériveurs à ancre flottante pistés par satellite ont été mises à l'eau à la limite nord présumée de l'aire du stock. Ces données ont servi à projeter les schémas de dérive de façon rétrospective et prospective, et donc les sources et destinations possibles des oeufs et des larves. En considérant le développement du stade le plus jeune (stade I) au stade le plus vieux (larves), nous avons observé une progression du nord vers le sud et du large vers les côtes dans les schémas spatiaux de la fraye, et une croissance continue du niveau global de dispersion à mesure que la progéniture avançait en âge. Les données ne confirment pas l'hypothèse selon laquelle les zones côtières ou hauturières représenteraient de simples sources ou puits pour les oeufs et les larves de morue. Le transport joue un rôle important mais hautement variable, qui est couplé à la distribution des adultes géniteurs et à la disponibilité de conditions ou d'habitats appropriés au moment où les jeunes commencent à se métamorphoser et à s'installer sur le fond.
[Traduit par la Rédaction]

Introduction

Ichthyoplankton surveys have provided insight into the location of spawning sites (O'Boyle et al. 1984), routes of dispersal (Ellertsen et al. 1981), and to a lesser extent the areas of greater survival or appropriate nursery areas (Suthers and Frank 1989) of several stocks of Atlantic cod (*Gadus morhua*). A particular asset of such surveys stems from the capacity to age ichthyoplankton in terms of days rather than months or years as is the case for juveniles and adults. As a result, inferences about the location of spawning or settlement can be more accurate because of the immediacy of the surveys relative to environmental time scales and to the overall life-span of the organisms sampled. Furthermore, a single survey may provide observations from several cohorts enabling evaluation of possible scenarios of the processes that lead to the observed spatial pattern.

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Atlantic cod on the Newfoundland Shelf spawn primarily during March through to July (Templeman 1981; Myers et al. 1993), although the latter authors presented evidence that more limited spawning may occur throughout most of the year. Much of the knowledge dealing with spawning location on the northeast Newfoundland Shelf (Northwest Atlantic Fisheries Organization (NAFO) areas 2J3KL) is based on two principal sources of information. Plankton surveys conducted in the late 1950s by Serebryakov (1967) indicated that substantial numbers of eggs were found near the shelf-break along the outer edges of the major banks. Templeman (1981) combined this information with observations of commercial fishing and adult stock distribution patterns to conclude that the major spawning areas were associated with the offshore core of the Labrador Current. Templeman went on to suggest that variations in transport could be an important factor determining the proportion of eggs drifting to nursery areas, which are located principally in coastal regions (Templeman 1981; Anderson et al. 1993). However, neither Serebryakov nor Templeman discussed the importance of the limited areal coverage of their surveys or the effects of pack ice on the distribution of fishing effort. A recent analysis of data collected during research surveys conducted between 1948 and 1991 suggested that spawning of cod might not be restricted principally to shelf-break

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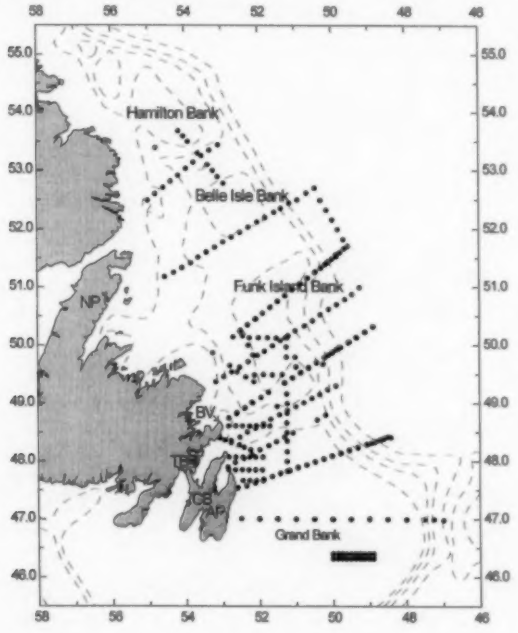
Fig. 1. The study area showing ichthyoplankton–CTD stations occupied during 1991–1992 and the bathymetry of the northeast Newfoundland Shelf. The 200-, 300-, 400-, and 600-m bathymetric contours are shown. The following coastal features are illustrated: AP, Avalon Peninsula; CB, Conception Bay; TB, Trinity Bay; NP, Northern Peninsula; BV, Bonavista.



areas but instead would occur over the entire shelf (Hutchings et al. 1993). These authors also suggested that inshore spawning might be of greater importance than previously anticipated in a manner consistent with Frost's (1938) documentation of extensive spawning by cod in coastal areas. Despite this analysis, an issue that remains unresolved is the relative contribution of the different spawning regions either to overall egg production or to settlement in the principal inshore nursery areas.

Serebryakov's (1967) observations of egg distribution have been combined to varying degrees with models of the circulation over the northeast Newfoundland Shelf to project the dispersal of eggs and larvae from their posited spawning sites (Templeman 1981; Helbig et al. 1992; deYoung and Davidson 1994). These theoretical treatments have proven disconcerting to some degree because the vast majority of particles are predicted to be advected to the southern limits of the system or off the shelf area completely where they would probably be lost from the population (Helbig et al. 1992). Mean flow and diffusion alone appear to be insufficient to generate the required cross-shelf exchange. Very few offspring can reach the major nursery areas along the coast unless one invokes the effects of highly stochastic events, such as storms or current instabilities, which increase horizontal dispersion but which would also appear to make reproduction a risky undertaking despite the apparent bet-hedging strategy used by highly fecund species. One therefore must question the accuracy of the fundamental life history of the stock (e.g., Hutchings et al. 1993) and our knowledge of the physical processes that influence dispersal on the northeast Newfoundland Shelf (Helbig et al. 1992).

Here we present the results of a study designed to extend our knowledge of spawning distribution as well as to gain a better understanding of the effect of variability in current structure on dispersal. The distribution of cod eggs and larvae is described from a series of ichthyoplankton surveys on the shelf with high cross-shelf resolution (20 km) and moderate along-shelf resolution (100 km). The mean and variance in surface currents were estimated from five experiments in which clusters of drogued drifters were released at the presumed northern limit of the stock's range. Biological and physical observations were combined into a drift model to (*i*) determine spatial and temporal patterns in the production of eggs, (*ii*) project patterns of drift and thus the ultimate destinations of eggs and larvae, and (*iii*) contrast predictions based on our observations of drifter behaviour with those made using more traditional, simpler advection–diffusion approaches. Our approach provides insight into the spatial structure of the advective variability of particles over the entire shelf as well as into the importance of short-term fluctuations in weather forcing.

Materials and methods

Ichthyoplankton sampling

Cruises were conducted during the periods of 1–15 June 1991, 6–20 May 1992, and 27 May – 12 June 1992. The cruise plan was designed to survey the northeast Newfoundland Shelf from approximately 47°N to 54°N. The sampling periods corresponded approximately to the midpoint of the spawning time for the 3L stock component (Myers et al. 1993). The survey plan called for seven cross-shelf transects with stations located approximately 20 km apart starting a few kilometres from shore and extending to shelf-slope break where water depth exceeded 500 m (Fig. 1). It was not possible to sample all transects during each survey because of variable ice cover. The most northerly region (Hamilton Bank) was sampled in 1991 and the most southerly region (the transect along 47°N and further south) was sampled in 1992. When pack ice prevented the occupation of stations along the entire length of a transect, sampling continued along the ice edge, at 20-km intervals, until progress towards shore was halted or until the next transect was reached.

Salinity and temperature profiles were made at each station to within 5 m of the bottom using a Seabird-19 or Seabird-25 conductivity–temperature–depth detector (CTD). The conductivity and temperature sensors of both instruments were calibrated under laboratory and field conditions.

Ichthyoplankton samples were obtained using a 4-m^2 Tucker trawl equipped with 1000-, 570-, and 333-μm mesh nitex. At each station, a single oblique tow of approximately 15 min was made at two knots (1 $m \cdot s^{-1}$). The net was lowered to 100 m at a rate of 0.6 $m \cdot s^{-1}$ and retrieved at 0.15 $m \cdot s^{-1}$. The net's position was monitored using a bridle-mounted Guildline model IV CTD. Volumes filtered were estimated from the average of two General Oceanics flowmeters positioned at the mouth of the net. Two flowmeters were used to avoid possible errors owing to malfunctions: abrupt changes in the difference in flow rates were considered to indicate blockage in one instrument. On deck the net was washed and samples were preserved in 2% buffered formaldehyde (1991) or 95% ethanol (1992).

Fish eggs and larvae were sorted and identified to the lowest taxonomic level possible by the Atlantic Reference Centre (Huntsman Marine Science Centre, St. Andrews, New Brunswick, Canada). Subsampling was performed using a beaker technique (van Guelpen et al. 1982) for samples in which numbers of a stage of a species exceeded 200 individuals. Eggs were staged according to the scheme of Markle

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Table 1. Description of the stages used in the classification of cod eggs based on the description of Markle and Frost (1985).

Stage	Description
I	From fertilization until the visible formation of an embryonic axis about the midgastrula
II	From the formation of an embryonic axis until the embryo is half way around the yolk, approximately the time of blastopore closure
III	From the end of stage II until the tip of the tail could reach the snout
IV	From the end of stage III until hatching

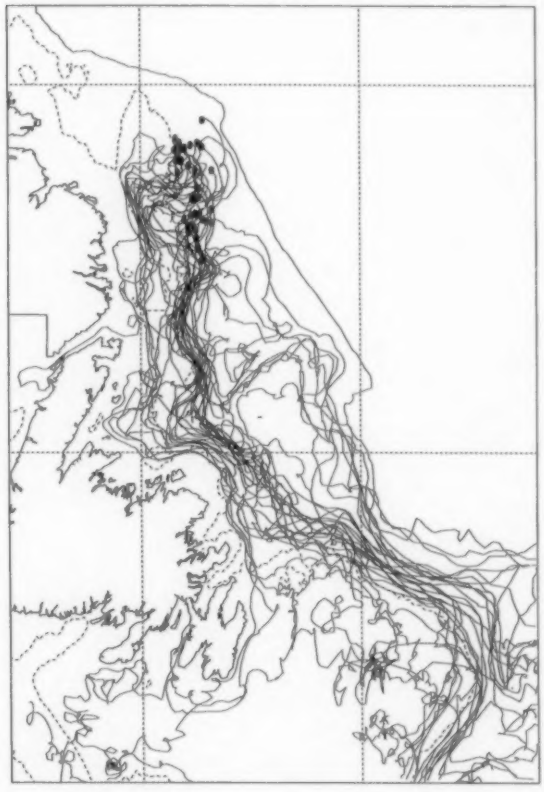
and Frost (1985) (Table 1). Although stage I–III eggs of cod cannot be distinguished from those of haddock (*Melanogrammus aeglefinus*) or witch flounder (*Glyptocephalus cynoglossus*) (Markle and Frost 1985), no late-stage eggs or larvae of the latter two species were caught in our surveys, suggesting that their spawning was either limited or did not occur during the study period (e.g., see Brander and Hurley 1992). Furthermore, surveys in coastal areas indicate that witch flounder spawn principally during late summer (Laprise and Pepin 1995; P. Pepin, unpublished data). Larvae were measured to the nearest millimetre for standard length using a dissecting microscope and a gridded background. Abundance (number/1000 m^3) was calculated for each egg stage and 1 mm larval length interval for each sample. Abundance estimates were corrected for the number of eggs or larvae that could not be staged or measured because of damage (<5%).

Drifting buoy data

Fifty satellite-tracked drifting buoys (manufactured by Seimac Ltd., Dartmouth, Nova Scotia) were deployed from a small fixed-wing aircraft near Hamilton Bank during 1992–1994. The buoys were spar shaped with an overall length of 0.89 m, mean and maximum diameters of 0.13 and 0.19 m, and an exposed height of 0.26 m. The requirement to be deployable through a small chute in the aircraft dictated the spar design. Each buoy was coupled to a 10 m long, 0.25 m diameter holey sock drogue with a compliant tether designed to absorb the majority of the surface wave orbital energy. The ratio of drogue to buoy–tether areas was 43:1, insuring a small windage effect (estimated to be a few centimetres per second at most), and the drogue was centered at 20 m to sample the upper layer of the ocean. Vertical positioning of the drogue was chosen to sample currents in a region representative of the median (Sundby 1983; Page et al. 1989) or peak concentrations of eggs and larvae (Anderson and deYoung 1995). Furthermore, the drogue would span depths at which significant gradients in density and shear may occur during summer months (Petrie et al. 1991), thus providing a representative sample of the current regimes influencing eggs and larvae as their vertical position varies during development (Page et al. 1989; Anderson and deYoung 1995). Each buoy was tracked by System Argos, and useful data were obtained from 41 of the 50 buoys deployed (some for periods exceeding 300 days, albeit much from the deep ocean off the shelf).

In normal years, much of the shelf is ice covered during the spring and early summer, so that buoys cannot be deployed during the egg–larval drift season. The period of 1992–1994 was no exception, and the ice cover was, in fact, heavier than normal (Colbourne et al. 1997). This limitation coupled with the need to gain small-spatial scale information from the buoys for other studies dictated our deployment strategy. The Hamilton Bank area was chosen as the deployment site because it was upstream of the general survey region and because existing knowledge suggested that it was an important spawning zone for cod (Templeman 1981). Other considerations included the desire to avoid entrainment into the offshore branch of the Labrador Current, since previous deployments indicated a very high likelihood that once the buoys were within the main branch, they would remain there until exiting the Labrador Current system into the eastward flowing North Atlantic Drift (Petrie and Isenor 1984; Helbig and Brett 1997). In 1991, half of the buoys were deployed in three nearly identical experiments during autumn (*i*) to sample the effects of storms that Helbig et al. (1992) argued could be important to the fate of eggs and larvae on the Newfoundland Shelf and (*ii*) to estimate small-scale dispersion. Consequently, the buoys were deployed in lots of 9, 9, and 7 in September, October, and November, just south of Hamilton Bank in diamond-shaped clusters about 30 km across centered on the 200-m isobath (Fig. 2). Data were returned from 9, 8, and 5 buoys, respectively.

Fig. 2. The trajectories of the 41 satellite-tracked drifting buoys that returned useful data in this study. The encircled asterisks denote the starting positions.



In 1992, we elected to sample surface currents during different density stratification regimes: one in summer when the mixed layer is relatively thin (typically ≤25 m) owing to fresh water input (primarily ice melt) and solar heating, and one in autumn after the onset of the storm season when the mixed layer is much deeper (typically >50 m). Buoys were deployed in two lots of 12 buoys each arrayed in a 60 × 75 km asymmetric diamond shaped pattern. The inshore-most buoys were seeded over Hamilton Bank proper (depth <100 m), while the offshore-most buoy was placed near the 600-m isobath close to what should be the core of the Labrador Current (Lazier and Wright 1993). A systematic failure of the buoys deployed in July delayed this part of the program until 1993, when deployments were made in July and November using buoys refurbished by the manufacturer.

Buoy positions were obtained from the Argos positioning system in near real time approximately 6 times daily. Detailed discussion and analysis of the drifter tracks are deferred to future work, but we note that the trajectories (Fig. 2) conform with general knowledge of the

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circulation (Petrie and Anderson 1983; Colbourne et al. 1997), and that no buoys were lost to the offshore branch before they encountered the northeast slope of the Grand Bank where the diffuse flow over the northeast Newfoundland Shelf converges and joins the offshore branch.

Our goal was to extract a statistically valid velocity field from the buoy data for use in the drift simulations; by valid we refer to accurate estimates of mean, variance, and temporal autocorrelation. Weighted B-splines were fit to the north–south and east–west coordinates of each buoy track to form evenly spaced, 3-h time series of position and velocity. The weights were chosen inversely proportional to the estimated quality of each positional fix as supplied by Service Argos (standard deviations of 150, 350, or 1000 m). The resultant series then were filtered with a seventh-order low-pass Butterworth filter to exclude variability at time scales less 10 h. Extensive numerical simulations with the velocity extraction algorithm indicated that the resultant speed estimates had errors of at most a few centimetres per second, the same size as those induced by the buoy's inherent inability to follow surface currents exactly (Helbig and Brett 1997).

Velocity estimates from all buoys were sorted into 50 km square bins and averaged to derive an overall Eulerian velocity field (Fig. 3). The number of degrees of freedom of each estimate was taken as the number of independent samples as inferred from the Eulerian integral scale (Garrett et al. 1985; Helbig and Brett 1997). Most bins on the shelf had at least 15 degrees of freedom; those in the inshore corridor preferred by the buoys had more than 60.

Drift model

Patterns of egg and larval drift were simulated by stepping the position of each tow station and its associated concentration of eggs and larvae forward or backward in time in the domain indicated in Fig. 3 in which the 600-m isobath serves as the offshore boundary. The drift model was based solely on the velocity fields estimated from the drifting buoys deployed in 1991 and 1993 and made use of both the mean currents as well as the estimated variances about those means. Each station was propagated in time using the simple, first difference model:

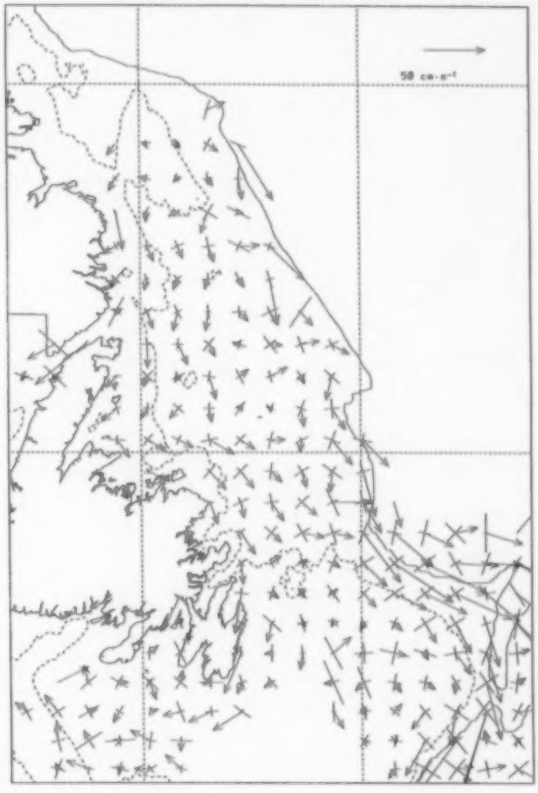
$$(1) \qquad \mathbf{x}(t + \Delta t) = \mathbf{x}(t) + \mathbf{u}(\mathbf{x}(t), t)\Delta t$$

The upper layer current $\mathbf{u}$, with east–west and north–south components (u, v), was split into the sum of the time invariant, spatially varying average flow, $\bar{\mathbf{u}}(\mathbf{x})$, (illustrated in Fig. 3) and a component, $\mathbf{u}'(\mathbf{x}(t), t)$, representative of the observed variability. The time step, Δt, was chosen as 1 h, more than adequate to resolve the most energetic scales of wind-induced motion and small enough to ensure numerical accuracy (Bennett and Clites 1987).

The simulations proceeded as follows. Each station and its egg–larval concentration were stepped forward or backward for a fixed time period 1000 times. At each time step, the mean velocity and variance were computed using bilinear interpolation of the four closest grid points. Particles striking the northern or southern boundaries and the 600-m isobath stuck there for the remainder of the run. Particles that struck the coastline were allowed to drift along the coast or move away from it: the normal velocity component was set to zero if it was directed onshore. At selected times, the total number of particles distributed during the 1000 runs were binned into 5 km square bins, and the resultant concentration fields were spatially contoured to provide an indication of the most likely source or fate of the observed, initial distribution. The total number of particles that drifted through the coastal zone, which we defined as the region within 50 km of the coastline, was also calculated.

The success of the simulations depended crucially on realistically mimicking the variability in currents, and we chose a simple statistical approach. The rotary spectrum of the horizontal currents sampled by the buoys over the northeast Newfoundland Shelf (Fig. 4) displays two frequency bands of importance. (In this representation, the fluctuating horizontal current is decomposed into a superposition of circularly polarized oscillations where positive (negative) frequencies

Fig. 3. Mean currents and variances as deduced from the drifting buoy trajectories. Variances are represented by a cross with its major axis oriented in the direction of maximum variance. The estimates represent space-weighted averages computed over a 50 km square grid. The 200- and 600-m isobaths are shown; the latter serves as the offshore boundary for the drift model.



correspond to anticlockwise (clockwise) rotation). The first band, which peaks at $f = -0.65$ cycles/h, represents clockwise-rotating inertial currents with periods centered on the inertial period of about 15.5 h and contains approximately 16% of the observed variance. Of greater importance are perturbations in the weather band with periods ranging from about 4 to greater than 15 days, which account for 59% of the variance. Both weather-band and inertial fluctuations are modelled in this exercise.

Currents experienced by drifting particles in both bands are highly correlated in time and the drift model must reproduce this feature. Weather-band fluctuations have correlation times of about 5 days (Fig. 5). The simplest model that captures this behaviour is a first-order autoregressive (Markov) process,

$$(2) \qquad u'(t + \Delta t) = a_u u'(t) + \varepsilon_u(\mathbf{x}(t))$$

$$(3) \qquad v'(t + \Delta t) = a_v v'(t) + \varepsilon_v(\mathbf{x}(t))$$

in which the innovations ε_u and ε_v are drawn at each time step from zero mean, normal distributions with variances appropriate to the spatial position of the particle. This model was selected over higher order autoregressive models using a minimal final prediction error criterion and was fit to the observed data using the Levension recursion program supplied in Kay (1987). A single model with $a_u = a_v = 0.97$ fits the two components equally well. We do not have sufficient data to determine whether a_u and a_v vary spatially.

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Fig. 4. The rotary spectrum of currents sampled by the drifting buoys on the northeast Newfoundland Shelf. Positive (negative) frequencies represent anticlockwise (clockwise) rotating current constituents. Frequency is in cycles per hour (cph). The units of the spectrum are $m^2 \cdot h \cdot s^{-2}$ so that the area under the curve is proportional to the current variance. Confidence intervals are not shown, but the estimates have over 800 degrees of freedom on the basis of the number of independent samples computed with a 150-h decorrelation time (Fig. 5).

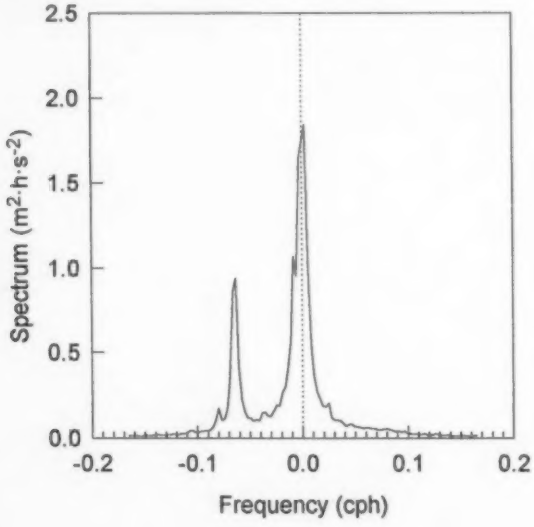
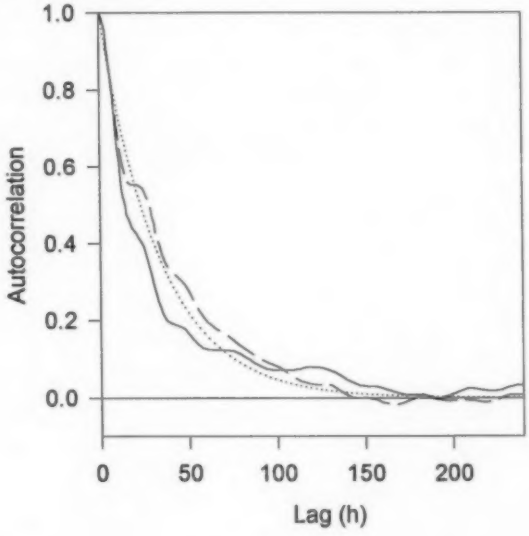


Fig. 5. The temporal autocorrelation function of currents sampled by the drifting buoys on the northeast Newfoundland Shelf. The north–south and east–west velocity correlations are represented by dashed and solid lines and the autoregressive model correlation is given by the dotted line.



In this mode, time serves as a path variable for a drifting particle. As time increases and the particle moves along its trajectory, it experiences variations in currents because the underlying (Eulerian) current field varies with both space and time. Thus, the drifter data on which the model is based include not only effects owing to time-varying winds, but also any spatial variability displayed by transient eddies, whether they are generated by winds or internal instabilities. In particular, eddies with short spatial correlation scales would lead to short correlation times.

Inertial band variability, for which the physics are relatively straightforward compared with the weather band, was simulated by randomly drawing a subseries of appropriate length from an artificial 25-year inertial current time series. This series was constructed by forcing a simple Pollard–Millard upper layer current model (Pollard and Millard 1970) with hourly winds observed at Bonavista. The Large and Pond (1981) formulation was used for the wind stress, and the free parameters in the Pollard–Millard model were set to 25 m for the mixed layer depth and 120 h for the frictional decay time.

We asserted earlier that the quality of the simulations depends crucially on how the variability in currents is modelled. A simple illustration of this shows a pair of drift tracks, one using the above-described formulation and one in which the variability was simply represented as a white noise process of appropriate variance (Fig. 6). The tracks are strikingly different and demonstrate how perturbations correlated in time can be effective in driving particles into regions of differing currents, whereas uncorrelated noise is ineffective because the overall excursion from the mean trajectory is too small compared with the length scales of variability of the mean flow (or the bin size of the model). This example also demonstrates that inertial currents are ineffective in deflecting a particle from its mean path; its time scale of 8 h is too short to effect significant change.

It is useful to put the above illustration into the context of effective diffusion. Classical turbulence theory (Taylor 1921; Tennekes and Lumley 1972) gives the effective diffusivity K_e as the product of the velocity variance and the Lagrangian integral time scale T_L defined as

$$T_L = \int_0^\infty R_u(\tau)\mathrm{d}\tau$$

where $R_u(\tau)$ is the Lagrangian velocity autocorrelation function. For the white noise (wn) case, $T_L = \frac{1}{2}\Delta t$ and $K_e^{wn} = \frac{1}{2}\mathrm{var}(u)\Delta t$, whereas for the AR(1) model, $T_L = (1 - a_u)^{-1}\Delta t = 33$ h and $K_e^{AR} = \mathrm{var}(u)(1 - a_u)^{-1}\Delta t$. The ratio of effective diffusivities is thus

$$\frac{K_e^{AR}}{K_e^{wn}} = \frac{2}{1 - a_u} = 67$$

The white noise model is Markovian in the displacements whereas our model is Markovian in the velocity but correlated in displacement, thus leading to a many-fold increase in dispersion. Typical values of K_e over the shelf computed with $T_L = 33$ h are in the range of $1–3 \times 10^{-3}$ $m^{-2} \cdot s^{-1}$, somewhat larger than the value of 1×10^{-3} deduced by Garrett et al. (1985) from iceberg drift on the Labrador Shelf.

Results

Egg and larval distributions

As indicated earlier, cod eggs were classified into four separate stages. The center of mass for each stage during the three offshore cruises followed similar patterns. Early stages of development (I and II) were found further south and offshore relative to the distribution of the later stages (III, IV, and larvae), which were found further north and closer to shore (Fig. 7). Results for the surveys conducted in 1991 and 1992 were combined because of the similarity in the center of mass and overall distribution patterns observed in the regions where sampling overlapped. Furthermore, the greatest concentrations of eggs and larvae were found in areas where sampling

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Fig. 6. Two backward projections of sample drift tracks simulated using correlated weather-band current fluctuations (left-hand trace) and using simple white noise (right-hand trace).

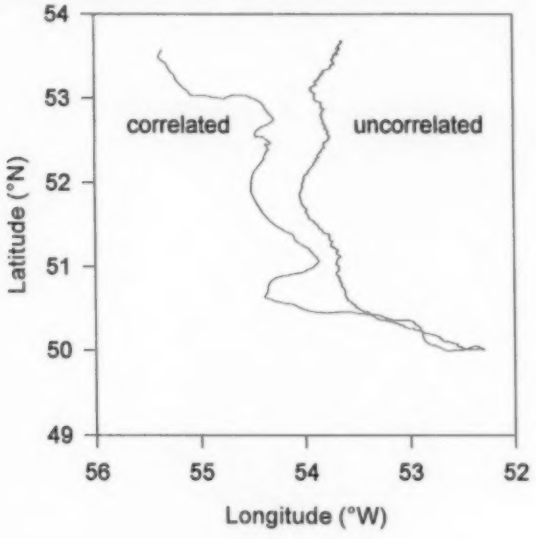
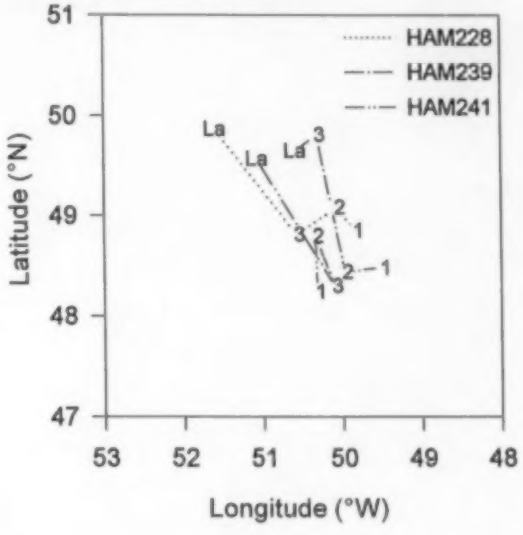


Fig. 7. Center of mass of the distribution of stage I (1) to III (3) cod eggs and larvae (La) during the three offshore cruises summarized in this study: HAM228 (1–15 June 1991), HAM239 (6–20 May 1992), and HAM241 (27 May – 12 June 1992). Data for stage IV were excluded from the figure because of the small number of specimens caught during the surveys.



overlapped during both years whereas relatively few specimens were caught in the northern-and southern-most regions. The spatial distributions of eggs together with that of larvae were contoured using SURFER (v. 5.01), which effectively eliminated variability at small spatial scales and thus damped much of the sampling variability inherent in ichthyoplankton surveys. The discussion here focuses on distribution patterns rather than absolute abundances.

Stage I eggs were most abundant near the shelf-slope break, but some were found on the central shelf southwest of Funk Island Bank (Fig. 8*a*). Stage II (Fig. 8*b*) and stage III (Fig. 8*c*) eggs showed relatively high concentrations across the entire width of the shelf in the region south of Funk Island Bank and along the northern edge of the Grand Banks, although their distribution was not restricted to that region alone. The few stage IV eggs caught during our surveys were almost entirely restricted to areas near the coast of Newfoundland or on the north-central region of the Grand Banks (Fig. 8*d*). However, caution should be exercised in generalizing our findings dealing with stage IV eggs as only 135 were caught during the study.

Cod larvae were the most widely dispersed stage (Fig. 9) and were caught from Hamilton Bank to the northern Grand Bank and from the shelf-slope break to coastal regions. The highest concentrations were found on the western side of Funk Island Bank. No substantial difference was found in the relative length frequency distribution of larvae in either the northern (50–54°N) or central (48–50°N) portions of our survey area, suggesting that the overall age distribution of larvae was relatively uniform across the entire shelf (Fig. 10). Few larvae were caught in the southern (≤48°N) portions of the survey area. Larvae of 4 to 5 mm standard length dominated the catch. Given the range of temperatures, egg development times, and sizes at hatch (Pepin et al. 1997), no growth in length during the period of yolk absorption, and an average growth rate of approximately 0.15 mm·day^{-1} (Bolz and Lough 1983; Gamble and Houde 1984), larvae sampled during our survey would be of an average age of 46 days post-spawning. Stage I through IV eggs would be an average of 4, 12, 19, and 25 days post-spawning.

Drift simulations

In this section, we present a number of forward and backward projections of the observed egg–larval horizontal distributions. Because the drift model contains a random component, the model is not invariant to time reversal and particles disperse irrespective of the direction in which the model is run. This means that the interpretation of the backward and forward runs is slightly different. In the forward runs, our aim was to predict the most probable spatial distribution at some time in the future (i.e., to answer the question of what the observed distribution of eggs and larvae would probably evolve into).

In the backward runs, we addressed the question of where eggs were most likely spawned. Ideally, we would have solved the optimization problem of determining the egg–larval distribution that when used to initialize the forward drift model led to the best fit between observed and predicted distributions. This is a difficult problem, however, and one that may have more than a single solution (depending on the goodness of fit criterion). We adopted the much simpler approach of running the drift model backward in time and allowing dispersion inherent in the model to represent natural environmental variability. The validity of this approach was checked by running the predicted initial distributions forward in time to see if they led to distributions similar to the original observed ones, which they did.

It it important to recognize that the distributions generated

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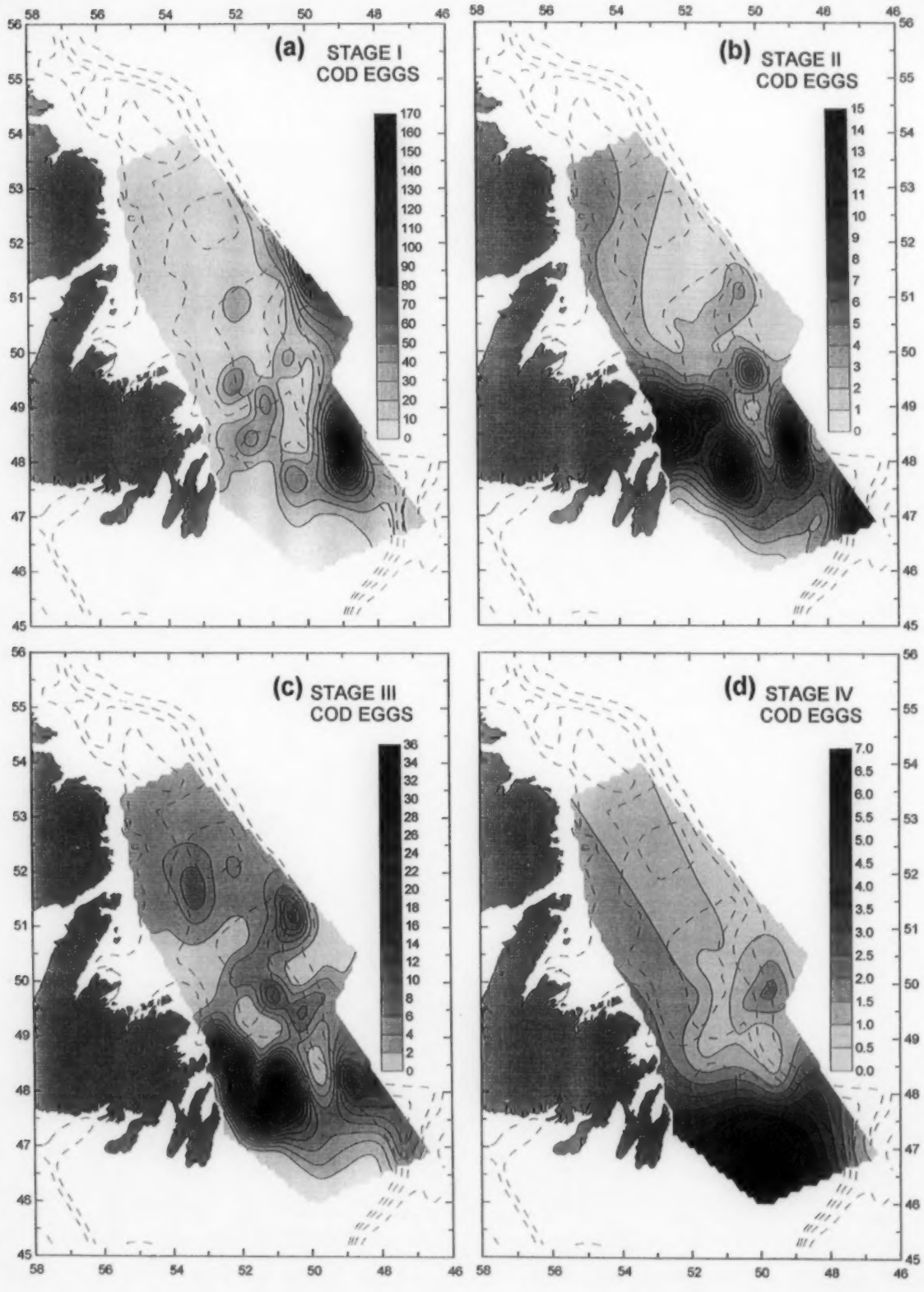

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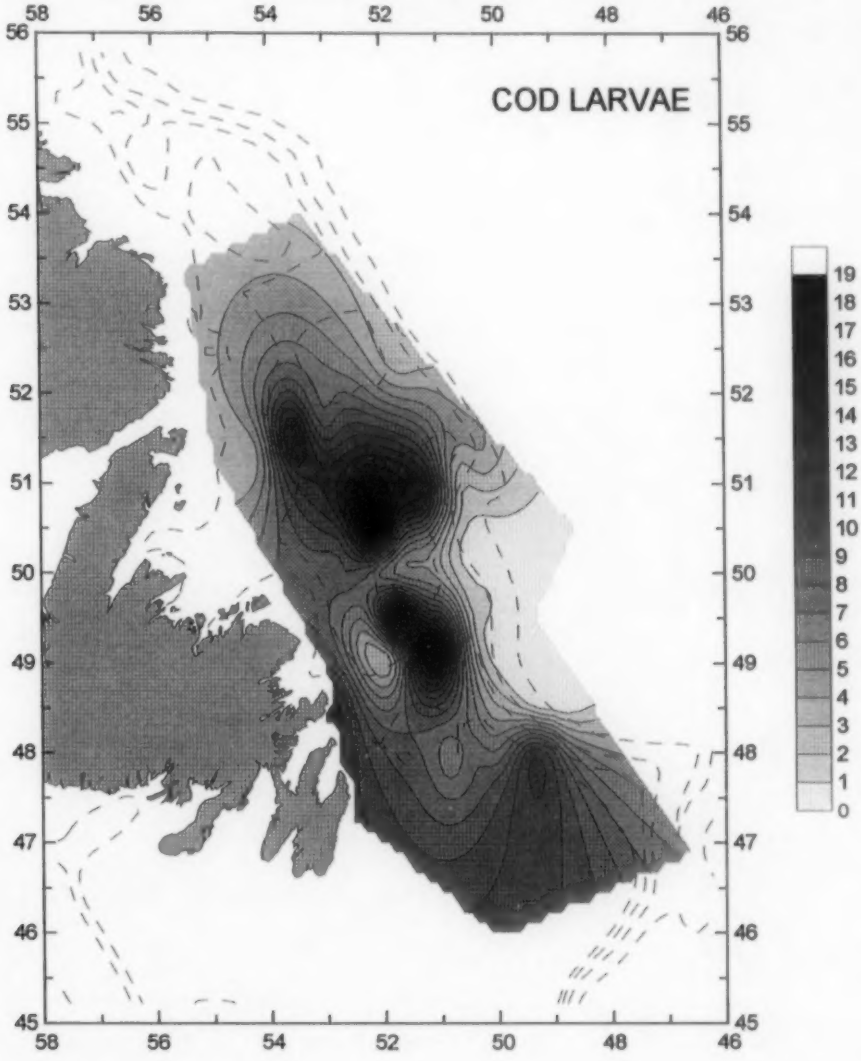
Fig. 8. Distribution of the areal density of cod eggs observed during the 1991–1992 surveys: (*a*) stage I (age 1–8 days), (*b*) stage II (age 9–16 days), (*c*) stage III (age 17–22 days), and (*d*) stage IV (age 23–28 days). The shaded scale bar defines the contour levels in terms of individuals per 1000 m^3. The dashed lines denote the 200-, 600-, and 1000-m isobaths.

Fig. 9. Distribution of areal density of cod larvae (age 40–52 days) observed during the 1991–1992 surveys. The shaded scale bar defines the contour levels in terms of individuals per 1000 m^3. The dashed lines denote the 200-, 600-, and 1000-m isobaths.



in the backward runs were based entirely on observed current patterns and could not include any information on where spawning actually occurred. In other words, some areas indicated as possible spawning sites may, in fact, be completely spurious. Finally, it is important to remember that the simulations are weighted by catch; stations with high concentrations are more important than those at which few ichthyoplankton were caught.

Backward projections

For the sake of brevity we show backward projections for stage III eggs and larvae only because they serve to represent a thorough range of ages and distribution patterns and because stage I and II eggs (1–16 days post-spawning) would have had little opportunity to undergo extensive drift. The larval and stage III distributions were stepped backward from their mean age to spawning, 46 and 19 days, respectively.

The backtracked larval distribution at day –46 (Fig. 11) predicts the northern (upstream) region, near Hamilton Bank, to be the most important one for spawning. This region may extend north of the model domain, which is bounded by Hamilton Bank (54°N). The coastal zone and especially the northern

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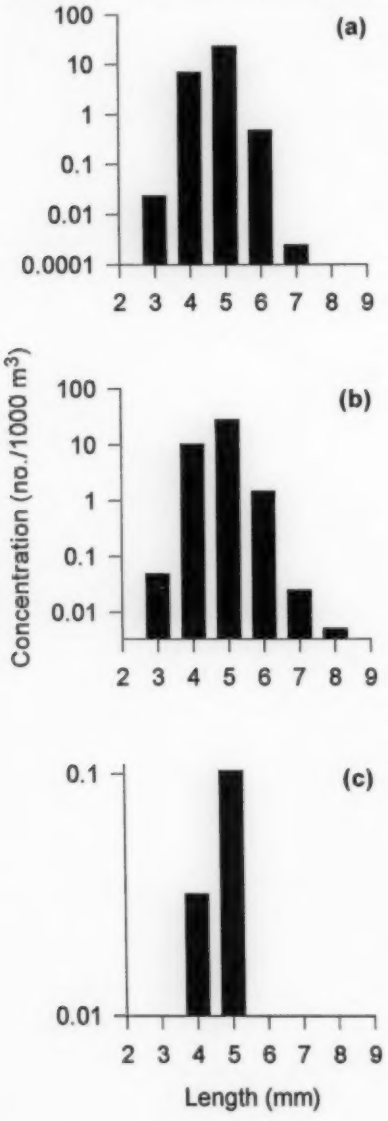
Fig. 10. Length-frequency distribution of cod larvae sampled in the (*a*) northern (50–54°N), (*b*) central (48–50°N), and (*c*) southern (≤48°N) portions of the survey area. Concentrations (number per 1000 m^3) represent the overall average abundance from all stations in each region (n = 115, 147, and 144 stations, respectively).



bays are also identified as significant, if not as spawning areas, then perhaps as way points for drifting eggs; active exchange between midshelf and the coastal zone is clearly indicated. This suggests that egg and larval production from regions outside the northern boundary for the stock (>55°N) may be a notable element in the stock-recruitment dynamics of the cod populations on the northeast Newfoundland Shelf, although our information currently prevents us from determining how far the source was along the Labrador coast.

The time series of snapshots of larval concentration demonstrates the existence of an inshore corridor in which particles spawned near Hamilton Bank were swept inshore and then alongshore to the southern limit of the northeast Newfoundland Shelf. The advection is nonuniform, of course, and concentration centers can change orientation over time. For example, the simulations predict that the Hamilton Bank region was most likely the source area of the large larval concentration observed seaward of Bonavista. By contrast, the simulations suggest that a portion of the more northerly Funk Island Bank concentration originated south of Hamilton Bank but was retained over Funk Island Bank for about 2 weeks, delaying its general southward drift. In fact, careful examination of detailed simulation plots shows a slow exchange of particles onto and off of the Funk Island Bank throughout the simulations. The coastal zone is also identified as a potentially significant area of eggs and larvae as fully 10% of the particles passed through it. These results are similar to those obtained from a simulation in which we seeded 1000 particles just south of Hamilton Bank (53°N, 53°30′W) and moved them forward for 46 days. This suggests that coastal areas were not important spawning areas for the observed larvae. Rather, they seem more likely to have served as way points for some fraction of the drifting eggs.

The spawning of stage III eggs is predicted to have been widespread across the shelf, but its center of mass is distinct and south of the larval centerpoint (Fig. 11), clearly implying a north to south spawning progression (Fig. 12). The inshore may be an important source for the observed nearshore stage III eggs as roughly 10% of the southern patch are predicted to have originated in the coastal zone. There may have been particularly high production associated with the western edge of Funk Island Bank, suggesting that the bank can serve as a retention zone for limited periods. While substantial production of eggs may have occurred on the northern Grand Banks, it appears that the stage III eggs sampled during our surveys were produced across the entire width of the northeast Newfoundland Shelf south of 52°N.

The backward projections for all early life stages were greatly influenced by the correlated noise structure used to simulate the fluctuating currents. To illustrate this point, we carried out another backward projection (Fig. 13) for larvae in which the noise was modelled as simple, independent white noise processes in u and v using the estimated variances appropriate to the drifting particle's position (Fig. 3). The predicted spawning distribution differs from that shown in Fig. 11. The white noise projections would suggest that spawning was confined principally to the inner portion of the Labrador Current, with almost no spawning having taken place along the shelf-slope break. The results of such simulations would have suggested a highly aggregated distribution of adult cod in spawning condition. In addition to lowering the estimated horizontal dispersion of particles, a white noise model would have resulted in a lower estimate of along-shelf advection of cod eggs. The predictions would have suggested that fewer offspring would have been produced from regions north of Hamilton Bank, the upper limit of the population's distribution. The contrast in projections between structured and random drifter behaviour is similar to the limitations by which Helbig et al. (1992) questioned the validity of simple advection–diffusion modelling of shelf circulation for the same region.

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Fig. 11. Time series of backtracked cod larva distributions at 4-day intervals. The contour lines indicate the relative distribution of organisms on a scale from 0 to 500 with an interval of 50.

Forward projections

Each egg or larval stage was stepped forward in time to an average age of 75 days post-spawning. Results are shown here for stage I (71 days), stage III (56 days), and larvae (29 days). Despite substantial differences in the initial distribution of each stage (Figs. 7, 8) in terms of both cross-shelf and along-shelf patterns, a number of similar features emerged in the projected distributions.

In all projections (Fig. 14) a significant proportion of the particles reached the eastern and southern boundaries of the model domain, indicating that substantial numbers of eggs and larvae were advected into the Labrador Current as well as onto or beyond the southern Grand Banks. In addition, there were substantial numbers of eggs and larvae that appeared to remain on the central portion of the shelf as well as on the northeastern edge of the Grand Banks. What contrasts with previous results (e.g., Helbig et al. 1992; Davidson and deYoung 1995) is the large number of particles that reached the coastal areas, notwithstanding the generally offshore distribution of stage I eggs. The highest concentrations were found along the eastern

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Fig. 12. Distribution of the areal density of stage III eggs backtracked 19 days to spawning. The contour lines indicate the relative distribution of organisms on a scale from 0 to 1000 with an interval of 50.

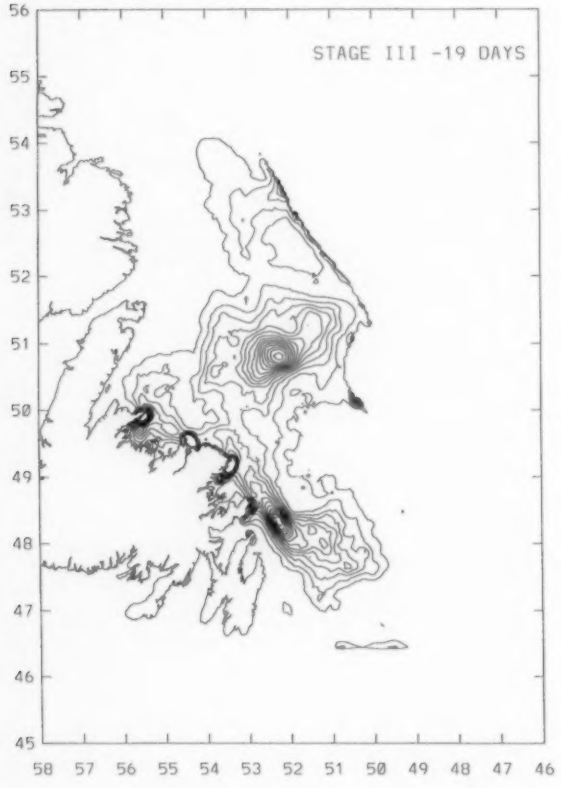
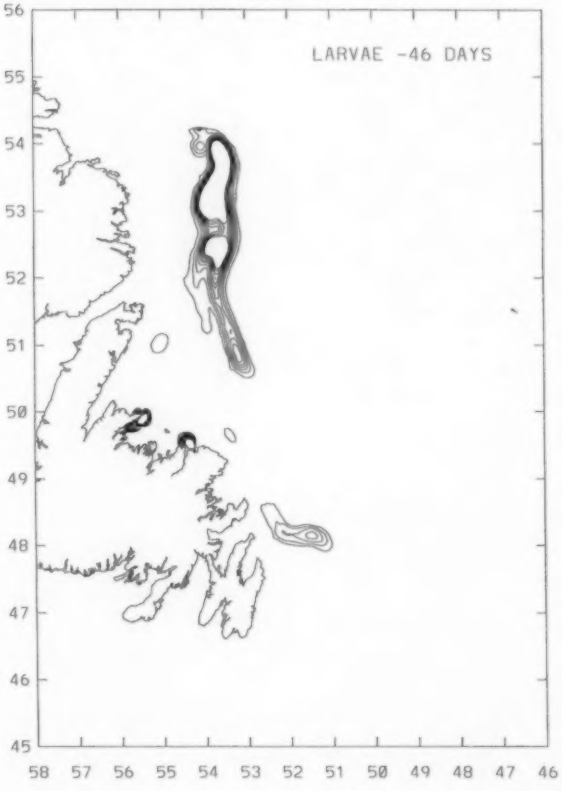


Fig. 13. Distribution of the areal density of cod larvae backtracked 46 days to spawning using uncorrelated weather-band noise. The contour lines indicate the relative distribution of organisms on a scale from 0 to 500 with an interval of 50.



shore of the Northern Peninsula as well as along the major headlands. Trinity and Conception bays as well as the Avalon Peninsula also appeared to be areas of high concentration but this may be due partly to local spawning in a region of minimal currents. The passage of particles through these regions increases the possibility that they will be retained or settle there. We caution, however, that the drift model and the currents upon which it is based do not resolve coastal circulation features, making it improper to overinterpret the predicted coastal distributions.

With the possible exception of the northern edge of the Grand Banks, the forward simulations identify no major concentration of larvae with major topographic features (e.g., Funk Island Bank). This result is in marked contrast with conditions that exist in the Scotian Shelf (O'Boyle et al. 1984) and Gulf of Maine (Werner et al. 1993), where strong topographic steering helps in the retention of particles over several major banks.

For brevity, we do not show the results of forward projections using uncorrelated anisotropic diffusion, but note that both horizontal dispersion and along-shelf advection were substantially reduced from the results shown here. Furthermore, a substantially greater proportion of particles were projected to have moved to the model's boundary at the core of the Labrador Current. Virtually no particles remained on the shelf and the dispersal to coastal areas was limited to a concentration of particles in Conception Bay and the southern edge of the Avalon Peninsula. White noise projections indicated that no particles would settle in coastal areas north of 48°N.

Discussion

At the inception of this study, a number of ideas were current regarding the reproductive strategy of Newfoundland cod and to what extent the environment acted as a mediating factor (Helbig et al. 1992). Some of these enjoyed the imprimatur of the scientific literature (Templeman 1981), while others assumed a more informal, but nonetheless real, authority among many fisheries scientists (Lear 1986). These notions included the concept advanced by Templeman (1981) that cod spawn principally on the flanks of the several banks that punctuate the Labrador and Newfoundland shelves, and that the offshore branch of the Labrador Current carries eggs and larvae downstream to suitable inshore nursery grounds. Serebryakov's (1967) egg and larval distribution maps were also widely interpreted in terms of preferential spawning near the banks, and this notion became pervasive amongst some biologists, as Hutchings et al. (1993) aptly point out, despite evidence in

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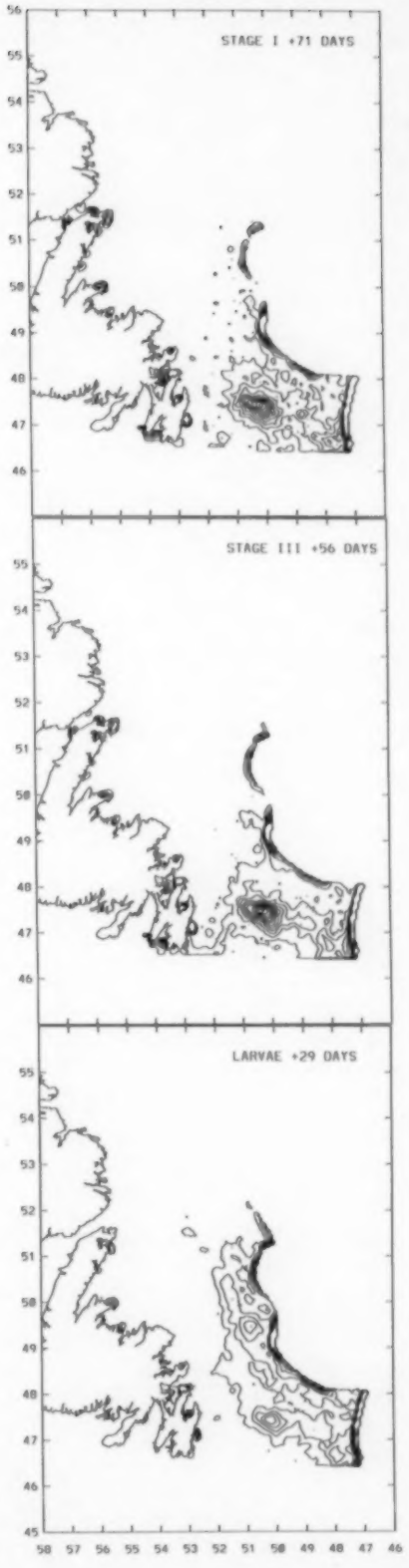

Fig. 14. Distribution of (*a*) areal density of stage I eggs stepped forward 71 days, (*b*) areal density of stage III eggs stepped forward 56 days, and (*c*) areal density of cod larvae stepped forward 29 days, all to a mean age of 75 days. The contour lines indicate the relative distribution of organisms on a scale from 0 to 500 with an interval of 25.

earlier literature that nearshore or more widely spread spawning was important (Frost 1938). There remains some debate as to the relative importance of spawning in coastal and offshore regions in terms of their contribution to the overall settlement and recruitment into nursery areas (Hutchings et al. 1993). Of course, a critical element missing from the debate was an extensive and systematically collected data set on egg and larval distributions and the attendant current fields. Such a set now exists. To what degree does it close the debate? To what degree are additional data still required?

The distribution patterns displayed distinct consistency. Eggs were widespread but there was an indication that there was transport along an inshore corridor in a manner consistent with the pattern observed using the drifting buoys (Fig. 3). Substantial concentrations of eggs were found near Funk and Belle Island banks (Fig. 8) and in a zone extending seaward from Bonavista. The distribution of larvae (Fig. 9) was drastically different. Most prominently, their center of mass lay upstream of that for the egg distributions, and it is clear that the distribution patterns of the egg stages would have been very unlikely to evolve into a pattern similar to that observed for the larvae. Considering the development from the youngest (stage I) to the oldest stages (larvae), we observed a south-to-north and an offshore-to-inshore progression in spatial distribution patterns as well as an increase in the overall level of dispersion with increasing age of offspring, with the possible exception of stage IV eggs, which were relatively scarce throughout the surveys. Both the observations and simulations in this study are consistent with some suggestions from other studies of cod reproduction in the Newfoundland region that were based principally on observations of adult characteristics. The egg and larval distributions observed in this study indicate that older stages of development are found further upstream. This supports Templeman's (1981) and Myers et al.'s (1993) studies, which indicate that spawning begins at the northern range of the stock and progresses southward with time. In addition, the observations and backward simulations indicate that spawning may occur over the entire shelf from the coast to the core of the Labrador Current. This contrasts with Templeman's (1981) proposal that spawning is concentrated at the shelf-slope break but is consistent with Hutchings et al.'s (1993) findings based on the distribution of spawning adults caught in research surveys.

Templeman's (1981) treatise dealing with the structure of cod stocks on the Newfoundland Shelf also clearly indicated the potential for significant along-shelf mixing of stocks arising from the dispersal of ichthyoplankton. Templeman emphasized the importance of transport in the offshore branch of the Labrador Current into which the eggs and larvae were presumably released and hypothesized that stock mixing might be governed by the intensity of the current. The idea was that the offshore branch would carry eggs and larvae downstream where they could be carried onto the shelves by various diffusive agents. Our results do not rule out a role for the offshore

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branch, but we note that over-the-shelf spawning is a much more effective way to seed the northeast Newfoundland Shelf with eggs. We also note that earlier drift simulations using a similar mean field and a simple model for storm-induced transport (Helbig et al. 1992) suggested a degree of independence between the northern cod stock (NAFO areas 2J3KL) and the Grand Banks stock (NAFO areas 3NO) largely because the predominantly barotropic current was ineffective in crossing isobaths. Essentially, the current served as a barrier to stock interchange, a notion strongly supported by drifting buoy tracks available at that time (Helbig and Brett 1997). However, the information obtained from the drifters released in this study clearly shows that cross-shelf mixing and dispersal of particles has been substantially underestimated. Storm- and (or) eddy-induced current fluctuations (in the period of 2–15 days) are predicted to make a significant contribution to the cross-isobath displacement of organisms. The most striking example of this is seen in the forward projections for stage I eggs. Even though transport in the offshore branch of the Labrador Current acts as a sink for eggs, many particles are dispersed onto the northern portion of the Grand Banks. In our view, the spatial and temporal structure of currents over the shelf are the important elements in the study of the dispersal of spawning products, not transport in the offshore branch.

The simulations also imply that a dynamic interchange of material between coastal regions and the midshelf is a significant factor in determining where larvae ultimately settle. The predicted exchange is two way: eggs and larvae may be transported from the shelf into coastal nursery areas, eggs can be spawned in the bays and later move out onto the shelf, or eggs and (or) larvae may drift into the bays and be retained there for some time before drifting back out onto the shelf. These scenarios are all consistent with Hutchings et al.'s (1993) suggestion that inshore spawning may be of greater importance to stock dynamics than previously expected because inshore areas can act as both production and nursery zones. In the last scenario, the coastal zone would act not so much as a nursery ground, but more as a baffle or temporary way station. The simulations do not permit us to infer the proportion of offspring that originate in the offshore or coastal areas. Instead, our results can only suggest that a significant fraction (3–10%) of the offspring sampled in our surveys have passed within 50 km of the coast. The time spent within this zone depends heavily on the backcalculated source; Hamilton Bank, and areas further north, may represent important spawning grounds for eggs and larvae that transit through the coastal zone. Inshore spawning, which peaks somewhat later than our surveys (Frost 1938; Laprise and Pepin 1995), may also represent an important source of offspring in coastal areas, and some of the later stage eggs sampled on the shelf may have been released in coastal areas (Fig. 12). Although it is possible that bays may represent retention areas (deYoung et al. 1994), observations show that the advection of plankton into and out of these regions is highly variable in time and strongly correlated to wind forcing (Pepin et al. 1995) and very weakly, if at all, related to mean circulation patterns (J.A. Helbig and P. Pepin, unpublished data).

The simulations do not provide much support for the idea that the northeast Newfoundland Shelf banks act as retention zones either. While the observations indicate enhanced concentrations near the banks, it is more likely that particles are retained there only for relatively short periods. We estimated residence times over the full study area by seeding particles over the shelf, running 1000 simulations, and computing the average time spent within 100-km squares. Residence times over Hamilton, Belle Isle, and Funk Island banks are predicted to be about 10–15 days, far too short for the banks to effectively trap eggs and larvae. Residence times over the remainder of the northeast Newfoundland Shelf ranged from 5 to 10 days. These times are much shorter than those estimated by Loder et al. (1988) for other banks on the Atlantic continental shelf. Consequently, it seems more reasonable to regard the entire northeast Newfoundland Shelf as a retention zone. In this regard, we note that a directed swimming rate by larvae in the range of 0.01–0.03 $m \cdot s^{-1}$ could greatly increase their retention on the shelf (and on the banks, too) similar to the result reported by Werner et al. (1993) for haddock and cod larvae on Georges Bank and Browns Bank.

Finally, we note that many of our conclusions depend strongly on our parameterization of transport owing to weather-band current fluctuations as well as the quality of the surface current field. Since currents are known inexactly over the shelf, any set of simulations must contain a stochastic element. We have adopted the simplest possible model capable of reproducing the autocorrelated noise in the observations, and its use has predicted a much greater role for dispersion than that found in other studies (Helbig et al. 1992; Davidson and deYoung 1995). Mechanistically, the probability for particles to pass from one current domain to another is increased over that of models that employ noncorrelated noise; any well-constructed model should share this feature. However, as noted earlier, our stochastic approach means that the predictions must be interpreted probabilistically. The obvious alternative is to employ a numerical model with high spatial resolution, incorporating realistic temperature and salinity fields and driven by realistic winds. Indeed, this is a necessary approach if one wishes to model actual observations in a mechanistic sense. In this sense, there still remain many environmental data to be collected including winds, mixed layer depths, evidence of current instabilities, and information about the small-scale spatial structure.

In addition to any biases caused by wind slippage, current fields constructed from drifting buoy data are potentially subject to two other classes of error, one related to survey timing and the other to the inherent character of Lagrangian sampling. In the present case, none of the deployments sampled the region strictly synoptically, as a buoy typically required 50 days to drift from Hamilton Bank to the southern part of the northeast Newfoundland Shelf. The errors introduced are small provided that the estimates in each bin spanned a period long enough to sample several wind cycles, and that the wind statistics did not change significantly during the experiment. Given the relatively short duration of each buoy experiment, the latter criterion was easily met. The former was marginally fulfilled; samples in most bins covered at least 30 days, especially within the inshore corridor where some bins had data from more than 60 days. Since wind-forced variability is concentrated into the 4- to 15-day period band (e.g., Anderson 1986; Wright et al. 1988), estimates in most bins represented at least three storm cycles and more typically five. The second error type arises from the fact that buoys that are not initially deployed uniformly over the area of interest do not uniformly

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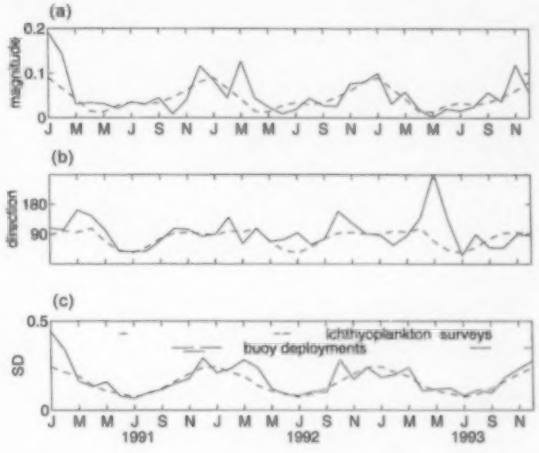
sample that area (e.g., Davis 1991). Buoys avoid zones of velocity divergence and can be steered by bathymetric features away from other areas downstream. It is clear from Fig. 2 that Funk Island Bank, for example, was poorly sampled. As mentioned, however, most bins were sampled with at least 10 degrees of freedom. The deployment strategy clearly was optimal for spawning near Hamilton Bank and less so for spawning on Funk Island Bank.

Of potentially greater concern is that the egg–larval surveys and deployments were carried out in different seasons, especially since an ice-covered sea responds differently to wind forcing than does the open ocean. There exists only weak evidence that the geostrophic currents vary seasonally over the shelf (Myers et al. 1989; Lazier and Wright 1993; Greatbatch et al. 1994). More important are fluctuations in directly wind-forced currents owing to seasonal and interannual differences in winds and ice cover. We analyzed 34 years of hourly wind data from the land station at Bonavista (see Fig. 1 for location) as a proxy for winds over the northeast Newfoundland Shelf, recognizing fully that winds measured at coastal sites are often correlated only weakly with winds offshore (Thomson 1983; deYoung and Tang 1989). Although other wind analyses are available for the North Atlantic (Thompson and Hazen 1983; Isemer and Hasse 1985), they do not cover the survey period and thus do not indicate how representative this period was. Like the other studies, we found distinct seasonal cycles in the mean wind stress and wind stress variance (Fig. 15). The analysis demonstrated that the period of 1991–1993 was remarkably normal in terms of wind forcing, although it was distinctly colder and marked by extensive ice cover (Colbourne et al. 1997). The eggs and larvae collected in the ship surveys drifted during the spring and early summer in a period of decreasing storm activity. By contrast, most of the buoys were deployed during the opposite phase of similar but increasing storminess. Overall, the buoys sampled somewhat more intense wind variability. On the other hand, egg–larval drift occurred in partially ice-covered waters, which respond more strongly to wind forcing than do open waters. The response depends on ice concentration and under-ice roughness (Fairall and Markson 1987; Anderson 1987; Guest and Davidson 1987) and is difficult to estimate. Without further study, we cannot evaluate the relative importance of the two factors.

Notwithstanding these limitations, this data set is the most extensive available of near-surface currents. Moreover, because the data were collected pseudosynoptically over the full shelf using a single instrument type designed to measure near-surface velocities, these data are arguably the best available for statistically modelling near-surface drift. We did not incorporate other drifting buoy tracks, because the buoys used in other studies were drawn from several designs and because few were drogued in the upper layer and most were not drogued at all (Helbig and Brett 1997).

To summarize, we suggest that it may be unrealistic to treat fixed coastal or offshore areas as sources or sinks for cod eggs and larvae on the northeast Newfoundland Shelf. Rather, the system appears to us as a dynamic one in which transport plays an important but highly variable role that is coupled to the spawning distribution of adult fish and the availability of suitable conditions or habitats when the young begin to undergo metamorphosis and settle. To return to the questions posed at the start of this section, we suggest that the debate is not completely

Fig. 15. Average seasonal cycle of monthly wind stress at Bonavista (dashed line) and observed monthly values during the 1991–1993 period (solid line): monthly mean wind stress magnitude (pascals, *a*), monthly mean wind stress direction (degrees true, *b*), and monthly standard deviation in wind stress magnitude (pascals, *b*). See Fig. 1 for location. The horizontal lines in *c* show the timing of the ichthyoplankton surveys and the buoy deployments, respectively.



resolved and that its ultimate resolution will require collection of information pertaining to the relative importance of these two issues. Furthermore, it is likely that the whole of the northeast Newfoundland Shelf is important to larval survival and that the system is coupled to the Labrador Shelf in the north and the northern Grand Bank in the south. However, it is apparent from our work, as well as from Templeman's (1981) conclusions, that the distribution of spawning fish is a highly important factor in determining the dispersal of young fish to potential nursery (or settlement) areas. Juvenile production for the northern cod stock (NAFO areas 2J3KL) may be linked to spawning in the northernmost and coastal areas of the stock's distribution. Eggs and larvae spawned along the northern edge of the Grand Banks (NAFO area 3L) may be more likely to settle in areas further south. Stock-recruitment relationships may therefore require consideration of variations in spatial and temporal features of both the environment and the adult population(s).

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Introduction to papers from the GAP VI workshop: effects of physical forcing on primary production processes in inland and marine environments

Richard D. Robarts

The Group for Aquatic Primary Productivity (GAP) was established in 1980 at the Societas Internationalis Limnologiae's (SIL) 21st Congress in Kyoto, Japan. GAP is a working group of both SIL and INTECOL (The International Association of Ecology) and is run by an international committee composed of seven scientists from Australia, Canada, Denmark, France, Israel, Japan, and the United States. The purpose of GAP is to assess the state of knowledge on a wide range of topics related to marine and freshwater primary production, to perform joint field and laboratory experiments using different techniques to test their comparability and reliability, to define major knowledge gaps and urgent research needs, and to publish these results in international journals.

To carry out its mandate, GAP holds workshops approximately every 3 years, and each workshop addresses a specific theme. In addition, GAP also convenes smaller ad hoc workshops to address a specific issue or to investigate a regional aspect of primary production. An example of the latter was the investigation of the warm-core Cyrus Eddy in the Levantine Basin of the eastern Mediterranean Sea, organized by Tom Berman of the Israel Oceanographic and Limnological Research Ltd. GAP depends upon proposals from interested scientists, who volunteer themselves and an organizational team and their institutions to organize and host workshops. Past workshops have been held in several European countries and Israel, and results have been published in several journals.

In 1993, GAP VI was held at Environment Canada's National Hydrology Research Institute in Saskatoon, Saskatchewan, a location that provided easy access to a wide variety of saline lakes and to the South Saskatchewan River. The theme of GAP VI was "Effects of physical forcing on primary production processes in inland and marine environments." The global concerns about the depletion of the ozone layer and the effects of increased ultraviolet (UV) radiation on aquatic systems resulted in many of the GAP VI participants examining this topic.

Four papers containing data produced during GAP VI appear in this issue of the *Canadian Journal of Fisheries and Aquatic Sciences*. Each paper was reviewed by two or three referees before being accepted for publication. I thank these referees for their time and interest in helping the authors prepare their final manuscripts.

Beardall et al. reported changes in respiratory activity and photosynthetic carbon assimilation of a cyanobacterium and a green alga when exposed to different levels of photosynthetically active radiation (PAR) and UV-A + UV-B radiation. The organisms differed in their responses, which led Beardall et al. to conclude that there is considerable interspecific variation in the response of autotrophic organisms to UV radiation and these responses are modulated by the level of concomitant exposure to PAR.

Information on UV radiation effects on the plankton of prairie saline lakes in Canada is lacking. Two studies were conducted with water from Redberry Lake, an oligotrophic saline lake. Maske and Latasa presented data on changes in phytoplankton pigment content, and suggested that environmental factors other than UV-B, or the state of cellular adaptation, partially controlled reduction of pigment. Ferreyra et al. measured the effects of UV-B on phytoplankton carbon assimilation, chlorophyll *a* concentration, bacterial numbers, and bacterial electron transport system activity, and found different responses to UV-B in these two groups of organisms.

In addressing the major GAP objective of conducting methodological comparisons, Bott et al. worked in the South Saskatchewan River to compare four methods to measure periphyton metabolism in in situ chambers. As a result of this research the authors were able to make recommendations for future research that is needed when measuring periphyton metabolism in chambers.

GAP workshops offer an unique opportunity for aquatic scientists to come together in short-lived, tightly focused teams and effectively address key research questions, as demonstrated by these papers from GAP VI.

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The effects of ultraviolet radiation on respiration and photosynthesis in two species of microalgae

John Beardall, Tom Berman, Stiig Markager, Rosa Martinez, and Vivian Montecino

Abstract: The effects of ultraviolet (UV) radiation (310–400 nm) on respiratory activity and photosynthetic carbon assimilation of the cyanobacterium *Aphanizomenon flos-aquae* and the green alga *Selenastrum capricornutum* were examined. When exposed for 2 h to 100 or 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) supplemented with UV-A + UV-B radiation, the two organisms differed in their responses. Photosynthetic rates were depressed by 33–81% in *A. flos-aquae* but never by more than 25% in *S. capricornutum*. Rates of oxygen uptake by *A. flos-aquae* following UV exposure were similar to or less than rates achieved without UV treatment. In contrast, *S. capricornutum* exhibited a stimulation of oxygen consumption immediately after exposure to UV + high PAR compared with treatment with PAR alone. In *A. flos-aquae*, dark carbon losses were completely eliminated after the cells had been exposed to UV (irrespective of the levels of PAR). This effect was less marked in *S. capricornutum*. Respiratory electron transport chain activity in *A. flos-aquae* was initially stimulated following UV + PAR exposure but was slightly impaired in *S. capricornutum*. The results indicate that there is considerable interspecific variation in the response of autotrophic organisms to UV radiation and that these responses are modulated by the level of concomitant exposure to PAR.

Résumé : Nous avons examiné les effets du rayonnement UV (310–400 nm) sur l'activité respiratoire et l'assimilation photosynthétique du carbone chez la cyanobactérie *Aphanizomenon flos-aquae* et chez l'algue verte *Selenastrum capricornutum*. Après exposition de 2 h à un rayonnement photosynthétiquement actif (RPA) de 100 à 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, avec complément de rayonnement UV-A + UV-B, les deux organismes ont réagi différemment. La production photosynthétique a baissé d'un pourcentage de 33–81% chez *A. flos-aquae*, mais jamais de plus de 25% chez *S. capricornutum*. Les taux d'assimilation de l'oxygène par *A. flos-aquae* après exposition aux UV étaient similaires ou inférieurs aux taux obtenus sans traitement aux UV. Par contre, on observait chez *S. capricornutum* une stimulation de la consommation d'oxygène tout de suite après l'exposition aux UV avec RPA élevé, par rapport au traitement par RPA seulement. Chez *A. flos-aquae*, les pertes de carbone en phase d'obscurité étaient complètement éliminées après exposition des cellules aux UV (quels que soient les niveaux de RPA). Cet effet était moins marqué chez *S. capricornutum*. L'activité de la chaîne respiratoire par transport d'électrons chez *A. flos-aquae* a été au départ stimulée après l'exposition aux UV+RPA, mais a été légèrement entravée chez *S. capricornutum*. Les résultats indiquent qu'il existe des variations interspécifiques considérables dans la réaction des organismes autotrophes au rayonnement UV, et que ces réactions sont modulées par le niveau d'exposition concomitante au RPA. [Traduit par la Rédaction]

Introduction

Solar ultraviolet (UV (especially UV-B); 280–320 nm) radiation is recognized as having a broad range of direct and indirect effects on aquatic biota. These include a decrease in primary productivity of phytoplankton (Smith 1989; Cullen and Lesser 1991; Hader and Worrest 1991), benthic macroalgae, and seagrasses (Larkum and Wood 1993). UV radiation is also likely to bring about changes in phytoplankton species composition by differential direct effects on the algae (Smith et al. 1992; Helbling et al. 1992) and may cause damage to herbivores, which in turn would alter grazing pressures and thereby effect changes in community structure and dynamics (Karanas et al. 1981; Bidigare 1989; Karentz et al. 1994).

Although the highest levels of UV-B radiation are found in the tropics, depletion of stratospheric ozone is causing a significant rise in UV-B penetration in both Antarctic and Arctic environments (Crutzen 1992; Austin et al. 1992). The nature of solar UV radiation, its potential consequences to phytoplankton and primary productivity, and the resistance and recovery mechanisms found in algal cells have been thoroughly reviewed by Vincent and Roy (1993).

Most studies on the effects of UV radiation on the biology and primary productivity of aquatic plants have concentrated

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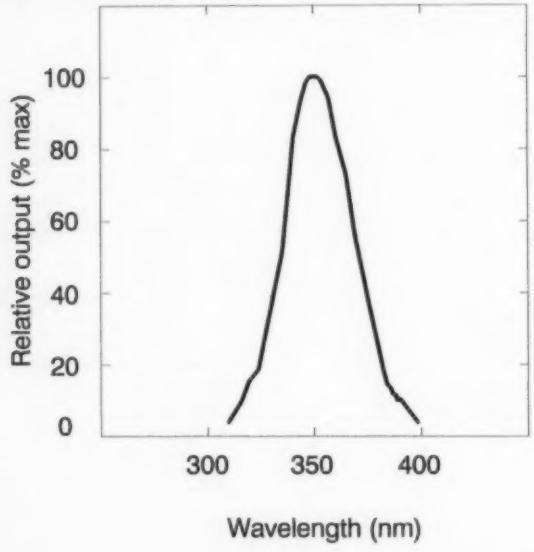
Fig. 1. Spectral output of the lamp (Toshiba FL20S BLB) used as a UV source in the experiments.



on the effects upon aspects of photosynthesis. Very little attention has been paid to the consequences of UV radiation to plant respiration, despite the fact that UV radiation can penetrate to biologically significant depths in clear waters (Vincent and Roy 1993) where lower levels of photosynthetically active radiation (PAR; 400–700 nm) would mean that respiratory losses could be a major determinant of net primary productivity. Moreover, in mixed water columns the development of phytoplankton spring blooms and areal primary productivity are sensitive to small changes in respiration rates (Smetacek and Passow 1990). Hence, overall effects of UV radiation on carbon fixation and respiration might be of equal importance for the ecology of phytoplankton.

Respiratory activity in algae has been most often measured directly as changes in dissolved oxygen (DO). Other methods have also been utilized to estimate planktonic respiration. The ETS (electron transport system) method has been used to estimate potential respiratory capacity of phytoplankton (Packard 1971; Owen and King 1975). This technique is based on the assumption that the activity of the respiratory ETS, as measured by the transfer of electrons to a tetrazolium dye, can be directly related to in vivo respiration, although as yet only a few studies have investigated the relationship between this method and direct DO measurements (Kenner and Ahmed 1975*b*; Packard 1971; Packard and Williams 1981; Martinez 1992).

Another approach to estimate algal respiration has been to follow the loss in the dark of ^{14}C from cells previously radiolabelled in the light. This method has been applied both in marine (Eppley and Sharp 1975; Smith 1977) and in freshwater environments (Berman and Kaplan 1984) but does not appear to have been used for evaluating respiration and release of dissolved organic carbon in algal cultures.

This paper describes experiments to investigate the effect of UV radiation upon both the photosynthetic and respiratory activities of two freshwater microalgae viz. *Aphanizomenon flos-aquae*, a cyanobacterium responsible for surface blooms in certain situations, and *Selenastrum capricornutum*, a green alga. Since the consequences of exposure to UV radiation can be modulated by the flux of PAR (Cullen and Neale 1993), we studied the effects of UV at different levels of PAR on photosynthetic carbon uptake, dark carbon release, respiratory oxygen consumption, and the respiratory ETS activity in these two organisms.

Materials and methods

Organisms and growth conditions

Selenastrum capricornutum (Freshwater Institute culture No. 107) and *Aphanizomenon flos-aquae* (Utex culture No. 1648) were grown in batch culture in a medium containing 25 μM $CaCl_2$, 150 μM $MgSO_4$, 100 μM $NaSiO_3$, 600 μM $NaHCO_3$, 150 μM KCl, 4 μM HEPES and trace minerals containing trinitriloacetic acid. $NaNO_3$ (1 mM) was added to the medium for *S. capricornutum*. Photon flux (PAR) for the cultures was 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (continuous light) with no exposure to UV radiation during growth. Temperature was maintained at 21°C. The pH was 7.6 for *S. capricornutum* and 7.1 for *A. flos-aquae*. Although the culture pH changed slightly with incubation at different photon fluxes, there were no appreciable changes with different UV exposures.

Experimental protocol

Cell suspensions (100 mL) were taken directly from cultures and incubated in plastic Whirl-Pak bags for 2 h at high (550 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 11 983 $\mu W \cdot cm^{-2}$), medium (350 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 6536 $\mu W \cdot cm^{-2}$), or low (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 3268 $\mu W \cdot cm^{-2}$) photon flux of PAR, supplemented (or not, in the case of controls) with UV-A + UV-B radiation. The lamp used for UV exposures (Toshiba Electric FL20S BLB) had a sinusoidal spectral output with a peak intensity at 352 nm, with lower and upper cutoffs at 310 and 400 nm, respectively (Fig. 1). The Whirl-Pak bags used for incubations are transparent to UV and, under the conditions of exposure employed, do not produce materials toxic to phytoplankton (Smith et al. 1992). PAR was determined with a Li-Cor PAR quantum sensor and UV radiation by means of UV-A and UV-B sensors (SD 104, MACAM Photometrics). The UV-A and UV-B sensors have a high sensitivity in a narrow peak at 360 ± 10 and 310 ± 10 nm, respectively, and cover bandwidths of approximately 100 nm (P. Bossard, Lake Research Laboratory, EAWAG, Switzerland, personal communication). Using these UV sensors we obtained values of 1000 and 370 $\mu W \cdot cm^{-2}$ for UV-A and UV-B, respectively, from a clear sky with a PAR reading of 1760 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (38 344 $\mu W \cdot cm^{-2}$). The rather high UV-B:UV-A ratio measured under these circumstances suggests that our UV-B sensor may have also been detecting some UV-A radiation. Furthermore, broad band-pass UV-A sensors tend to cut off wavelengths above 360–370 nm (Quesada et al. 1995). Consequently our estimates of UV-A may have been an underestimate. Precise conditions of PAR exposures differed between experiments and are described in the relevant figure or table. Measured values for the UV-B fluxes used in experiments were 224 ± 21 and 61.7 ± 2.5 $\mu W \cdot cm^{-2}$ for *A. flos-aquae* and *S. capricornutum*, respectively. Measured values for the concomitant UV-A fluxes were 444 ± 59 and 123.7 ± 4 $\mu W \cdot cm^{-2}$ for *A. flos-aquae* and *S. capricornutum*, respectively. A second, limited set of experiments on *S. capricornutum* exposed to the higher UV fluxes gave results essentially similar to those presented here.

Immediately after light exposure, replicated samples were taken for chlorophyll determinations and cell counts. Further replicate samples were taken twice during the subsequent incubations. For chlorophyll determinations, 3-mL aliquots of cell suspensions were collected on GF/C filters and extracted at 80°C for 5 min with 10 mL of 90% ethanol. Fluorescence of the sample before and after acidification was determined using a Turner Designs fluorometer. Chlorophyll

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Table 1. Cellular chlorophyll content (picograms of chlorophyll *a* per cell) as a function of time in darkness following treatment of cultures of *Aphanizomenon flos-aquae* and *Selenastrum capricornutum* with or without UV irradiation under three levels of PAR.

	Low PAR ($100\ \mu mol \cdot m^{-2} \cdot s^{-1}$)		Medium PAR ($300\ \mu mol \cdot m^{-2} \cdot s^{-1}$)		High PAR ($550\ \mu mol \cdot m^{-2} \cdot s^{-1}$)	
Time (h)[a]	+UV	–UV	+UV	–UV	+UV	–UV
			Aphanizomenon flos-aquae			
0	0.14	0.10	0.10	0.17	nd	0.15
3	0.15	0.17	0.15	0.08	0.12	0.24
22	0.22	0.10	0.19	0.19	0.12	0.24
			Selenastrum capricornutum			
0	0.41	0.35	0.44	0.30	0.23	0.28
3.8	0.37	0.29	0.30	0.22	0.32	0.32
22	0.25	0.38	0.21	0.28	0.26	0.31

Note: nd, not done.
[a]Hours in darkness.

concentrations (corrected for pheophytin) were calculated using the equations of Strickland and Parsons (1972).

For algal cell counts, 2- to 3-mL portions were fixed with Lugol's iodine and subsequently four replicates of each sample were counted under a light microscope in a Neubauer haemocytometer chamber.

^{14}C uptake in light and release in the dark

For each experiment, algal cell suspensions (24 mL) were placed in six Whirl-Pak bags to each of which 37 kBq $H^{14}CO_3$ was added. Immediately after addition of [^{14}C]bicarbonate, zero time and total activity samples were taken. The zero time samples were filtered and processed immediately (see below). Total added radioactivity in each bag was measured by adding 200 µL of the sample to 400 µL of Carbosorb and 5 mL Aquasol scintillation fluid (DuPont NEN). The total counts were used to check and correct for differences in added counts within the experimental bags.

The bags were exposed for 2 h to PAR with or without UV radiation (see above) after which duplicate, 10-mL samples were taken from two of the bags and the particulate fraction was collected on GF/C filters. Inorganic [^{14}C]bicarbonate remaining on the filters was removed by a 1-mL rinse with 0.5 M HCl. The filters were then left overnight before adding Aquasol and counting by scintillation to determine ^{14}C incorporation into particulate material. To evaluate extracellular release of ^{14}C-dissolved organic matter, duplicate 5-mL samples of the filtrate were placed in scintillation vials and acidified with 0.5 M HCl to approximately pH 2. After gentle shaking for 8–12 h in open vials to remove residual inorganic ^{14}C, a 1-mL portion of filtrate was mixed with Aquasol and the radioactivity assayed by scintillation counting.

Dark carbon losses from respiration and extracellular release were determined by incubating the remaining four bags in darkness after the 2-h light period. Duplicate 10-mL samples were taken from each of two bags and processed as described above after 4 and 22 h in the dark. Note that concentrations of inorganic carbon were not measured in these samples and therefore the results of ^{14}C incorporation experiments or loss are expressed as disintegrations per minute (dpm).

Details of the precise light exposures for each experiment are given in the appropriate figure or table captions.

Respiratory O_2 consumption

After exposure to PAR with or without UV irradiation, subsamples (2 mL) of cell suspensions were placed in the darkened chamber of a Hansatech DW1 oxygen electrode. Respiratory oxygen consumption was measured at various times (up to 22 h) after transfer of the cells to darkness. The temperature in the electrode chamber during measurements was maintained at 21 ± 0.1°C by means of a Lauda RMS6 refrigerating circulator. The electrode was calibrated using air-saturated water (283 nmol $O_2 \cdot mL^{-1}$) and sodium bisulphite (to give zero O_2).

Electron transport system activity

Activity of the respiratory ETS was determined by methods based on those of Packard (1971) and of Kenner and Ahmed (1975*a*). Subsamples (20 mL) from cultures treated with PAR and UV as previously described were collected on GF/C filters and the cells disrupted using a Potter homogenizer. ETS activity in these extracts was determined using the reduction of the tetrazolium salt INT (2-(4-iodophenyl)-3,4-nitrophenyl-5-phenyltetrazolium) as described by Packard (1985).

Results

Cellular parameters

Chlorophyll concentrations immediately after irradiation were very similar, within the same species (SE ±12%), for all experimental treatments in this study (Table 1). In *S. capricornutum*, there was a significant drop in cellular chlorophyll content after 2 h exposure to light, with the lowest pigment levels occurring at high light conditions. The addition of UV irradiation with low or medium PAR depressed cellular chlorophyll contents but not with high PAR. After 22 h in darkness, *S. capricornutum* that had been exposed to UV irradiation together with low, medium, or high fluxes of PAR showed chlorophyll values of 66, 75, and 84%, respectively, compared with cells not exposed to UV irradiation. The trend in *A. flos-aquae* was less clear, with no evident trends immediately after the radiation treatment or subsequent to the 22-h dark incubation.

^{14}C assimilation in the light and dark carbon loss

Photosynthetic rates of *A. flos-aquae* reached light saturation at approximately $300\ \mu mol \cdot m^{-2} \cdot s^{-1}$ ($6536\ \mu W \cdot cm^{-2}$) in the absence of UV irradiation (Fig. 2A). When UV irradiation was supplied with PAR, photosynthetic rates of *A. flos-aquae* were similar at all PAR photon fluxes and were strongly inhibited (33–81% compared with cells not treated with UV irradiation; Fig. 2C). In contrast, photosynthetic rates in *S. capricornutum* were similar at all PAR levels and were only slightly inhibited by UV irradiation (Figs. 2B and 2C).

In most experiments there was a loss of labelled carbon in the dark. For *A. flos-aquae*, the release was highest in the control (21.4% over 22 h) and was higher during the first 3 h of darkness than subsequently (Table 2). However, in contrast,

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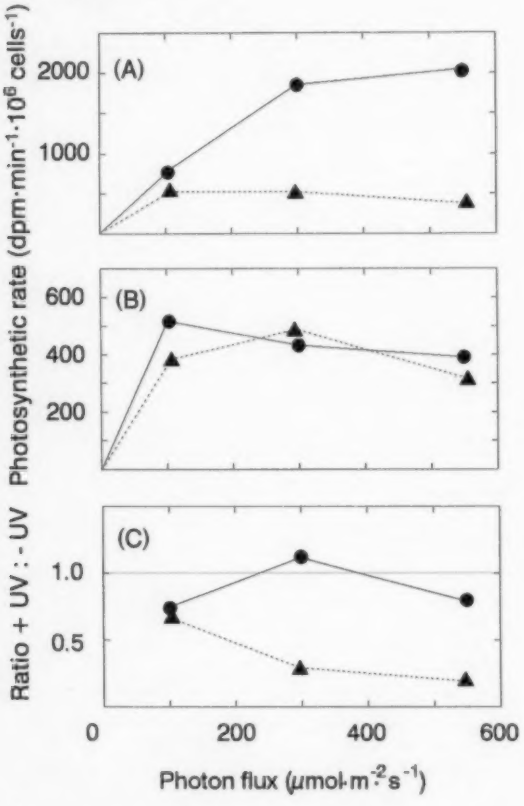
Fig. 2. Photosynthetic rates of (A) *Aphanizomenon flos-aquae* and (B) *Selenastrum capricornutum* as a function of photon flux. Photosynthetic rates are expressed as dpm·min^{-1}·10^6 cells^{-1}. Cells were exposed to PAR alone (●) or to PAR supplemented with 224 or 61.7 μW·cm^{-2} UV-B (▲) for *A. flos-aquae* and *S. capricornutum*, respectively. Exposure of *S. capricornutum* to the higher level of UV irradiation produced essentially the same result as shown here. (C) Ratio of rates obtained with different PAR levels of UV to that without UV for *S. capricornutum* (●) and *A. flos-aquae* (▲) ($n = 3$).



the UV-treated samples of *A. flos-aquae* showed a slight increase in net ^{14}C incorporated over 22 h, although in cells treated with both high and low levels of PAR there were perceptible drops in incorporated ^{14}C during the first 3 h of darkness (Table 2). With *S. capricornutum*, the UV-treated cells exposed to high and medium levels of PAR lost less ^{14}C than those without UV. The opposite occurred in cells exposed to low PAR + UV (Table 2).

In all instances, the proportion of released ^{14}C-labelled dissolved organic material in culture filtrates was negligible in comparison with dark carbon loss when this occurred (<2 and <1% of total fixed counts in *A. flos-aquae* and *S. capricornutum*, respectively). The levels of extracellular dissolved organic carbon released showed no effect of UV exposure or of different PAR fluxes.

Rates of oxygen consumption

Rates of O_2 consumption (measured directly by oxygen electrode) in *A. flos-aquae* were dependent on the photon flux of PAR given during the light exposure (Fig. 3) and showed a clear enhancement of postillumination respiration (EPIR; Falkowski et al. 1985) over the first 3 h of darkness in all experiments. When cells were previously exposed to PAR together with UV radiation, dark respiration and the EPIR effect in *A. flos-aquae* were decreased.

Rates of oxygen consumption by *S. capricornutum* also showed a strong EPIR after high light irradiation (Fig. 4A), although this was markedly decreased at lower PAR exposures. Initial rates of respiration decreased with previous PAR exposure (Fig. 4). In contrast to *A. flos-aquae*, EPIR in *S. capricornutum* was slightly stimulated by the presence of UV during exposure to PAR, and even cells exposed to low PAR (+ UV) exhibited an EPIR effect.

Electron transport system activity

In *A. flos-aquae*, cell-specific ETS activities increased during darkness. Exposure to UV + PAR resulted in a slight stimulation of ETS activity immediately after transfer of cells to darkness, whilst after 22 h in darkness, cells exposed to high PAR + UV had twice the ETS activity of cells exposed to PAR alone (Fig. 5).

In most cases, cell-specific ETS activity of *S. capricornutum* decreased during the 22 h in the dark, although under low PAR (± UV) values were higher after 3.8 h than initially. There were, however, no consistent effects of UV exposure (Fig. 5).

Curiously, the initial rates of oxygen consumption in *A. flos-aquae*, as determined by oxygen electrode, were significantly higher than ETS capacity (Fig. 6A). This was not the case after prolonged (22 h) exposure to darkness and did not appear to be related to UV exposure. In the case of *S. capricornutum*, only in the instance of cells exposed to high PAR + UV for 2 h did oxygen consumption exceed ETS capacity, and that only occurred during the first 5 min of darkness (Fig. 6B).

Discussion

The UV fluxes applied in these experiments were lower than the maximum fluxes generally observed at sea level from a clear sky. Thus, the effects that we observed on both carbon fixation and respiration were likely, if anything, to underestimate the impacts of UV radiation in near surface waters in nature, although we recognize that algae in a mixed water column are only exposed to high, surface irradiance for a short period of the day. Because of the characteristics of our UV-radiation sources, the algae in these samples were exposed to rather low levels of UV-B, and thus our results may not be directly pertinent to questions concerning the potential increase of UV-B owing to decreasing global ozone cover. Furthermore, the experiments we report were conducted in the presence of UV-A (with a UV-A:UV-B ratio of approximately 2), conditions under which UV-B inhibition of growth has been shown to be offset by repair mechanisms induced by UV-A (Quesada et al. 1995). Nevertheless, we have shown that UV irradiation affects not only photosynthetic capacity but also dark respiration in algae and that responses to irradiation may be species specific.

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Table 2. Effect of UV and PAR irradiation on dark carbon loss in *Aphanizomenon flos-aquae* and *Selenastrum capricornutum*.

	Low PAR (100 µmol·m^{-2}·s^{-1})		Medium PAR (300 µmol·m^{-2}·s^{-1})		High PAR (550 µmol·m^{-2}·s^{-1})	
	–UV	+UV	–UV	+UV	–UV	+UV
			Aphanizomenon flos-aquae			
4 h dark	14.7 (4.9)	13.1 (4.4)	13.6 (3.4)	–6.2 (–1.6)	11.1 (2.8)	8.5 (2.1)
22 h dark	20.7 (0.3)	–9.3(–0.5)	18.9 (1.0)	–15.3 (–0.8)	21.4 (0.6)	–25.8 (–1.91)
			Selenastrum capricornutum			
4 h dark	11.4 (3.0)	–1.0 (–0.3)	9.0 (2.4)	3.3 (0.9)	–7.5 (–2.0)	13.9 (3.7)
22 h dark	17.1 (0.9)	30.8 (1.6)	19.5 (1.0)	15.7 (0.8)	48.4 (2.6)	14.7 (0.8)

Note: Carbon losses for the indicated times are given as the percentage decrease of initial dpm in algal cells at the beginning of the dark period. A negative value signifies that ^{14}C assimilation increased during the first 3 h (or 3.8 h) darkness or the ensuing period. Values in parentheses are calculated rates given as the percentage change in the initial dpm per hour.

Fig. 3. Dark oxygen consumption rate of *Aphanizomenon flos-aquae* after a 2-h exposure to PAR with (solid bars) or without (grey bars) UV radiation. Conditions of PAR and UV exposure were as for Fig. 2. PAR fluxes were (A) 550 µmol·m^{-2}·s^{-1}, (B) 300 µmol·m^{-2}·s^{-1}, and (C) 100 µmol·m^{-2}·s^{-1}. Error bars are 1 SE ($n = 3$).

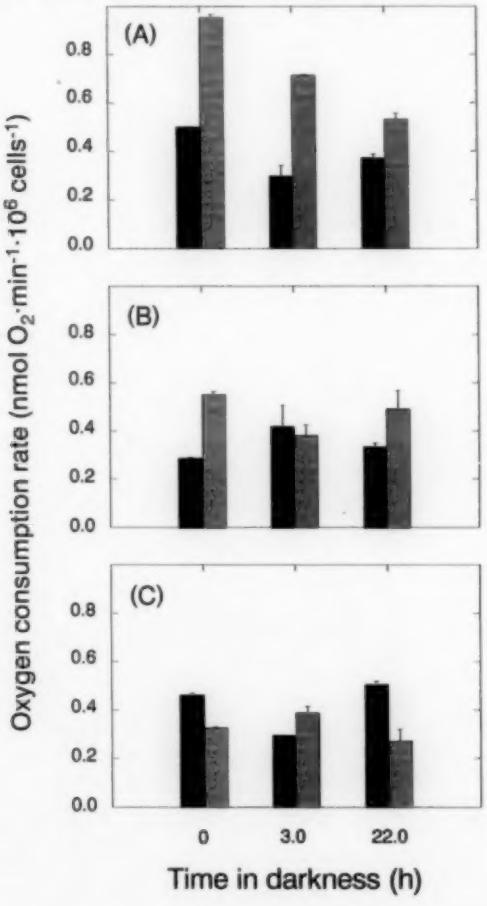

Fig. 4. Dark oxygen consumption rate (nmol O_2·min^{-1}·10^6 cells^{-1}) of *Selenastrum capricornutum* after a 2-h exposure to PAR with (solid bars) or without (grey bars) UV radiation. Conditions of exposure to PAR and UV were as for Fig. 2. Error bars are 1 SE ($n = 3$).

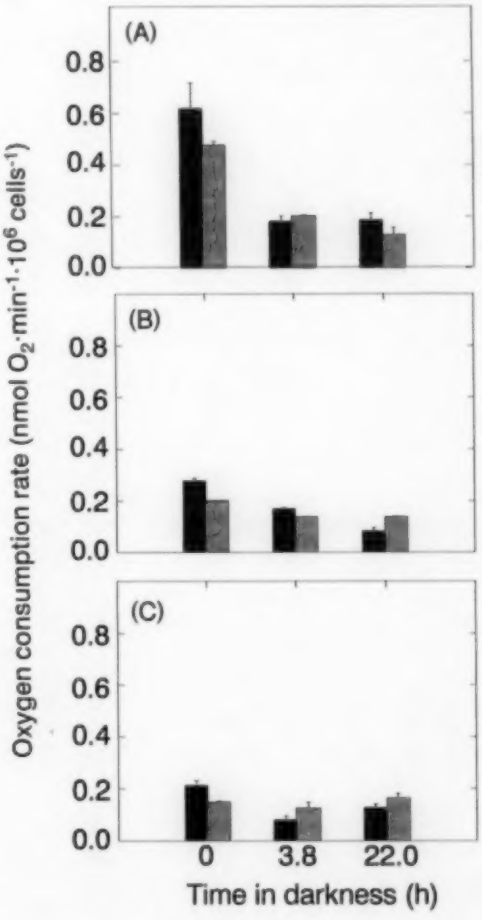

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Fig. 5. ETS activity of *Aphanizomenon flos-aquae* (A, B) and *Selenastrum capricornutum* (C, D) as a function of time following a 2-h exposure to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (A, C) or 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (B, D) with (solid bars) or without (grey bars) UV radiation. Conditions of UV exposure were as for Fig. 2. Error bars are 1 SE ($n = 3$).

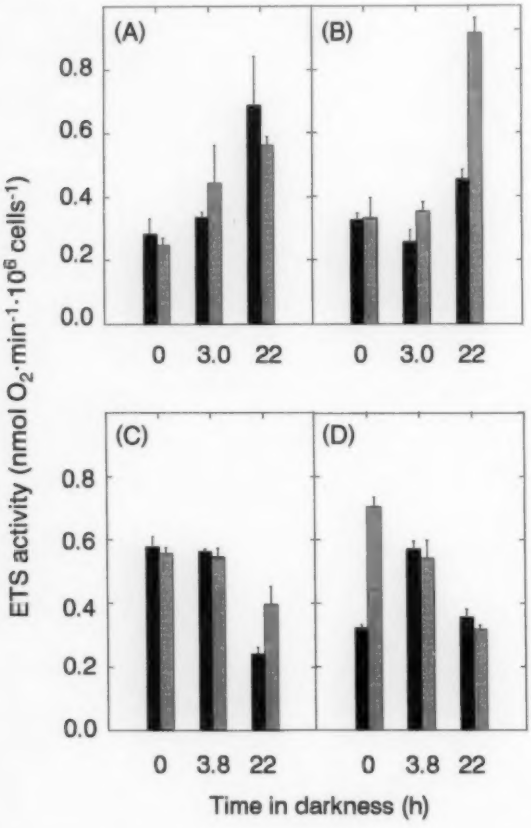
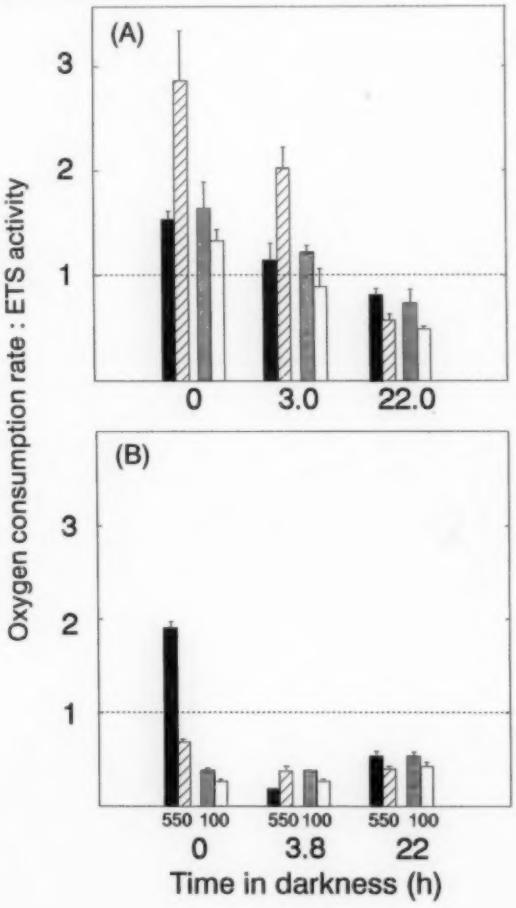


Fig. 6. Ratio of respiratory rate (O_2 consumption) to ETS capacity for *Aphanizomenon flos-aquae* (A) and *Selenastrum capricornutum* (B). Values are for cells exposed to 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (solid and diagonally hatched bars) or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (grey and open bars). Irradiation by PAR with or without complementary UV radiation is shown by the solid and grey or diagonally hatched and open bars, respectively. Note that in *S. capricornutum* only in one instance does respiratory O_2 consumption exceed ETS capacity, whilst in *A. flos-aquae* this occurrs in most samples, except after prolonged darkness. Error bars are 1 SE ($n = 6$).



Photosynthesis

In these experiments the influence on photosynthetic activity of UV irradiation combined with three intensity levels of PAR was determined only by incorporation of $^{14}CO_2$. The two species showed somewhat different responses to photon flux of PAR (Fig. 2). *Aphanizomenon flos-aquae*, as might be expected from an organism frequently associated with surface blooms in freshwater, required higher photon fluxes of PAR than *S. capricornutum* to attain saturation of photosynthesis. *Selenastrum capricornutum*, however, did show inhibition of photosynthesis by high fluxes of PAR alone. The two species also showed major differences in their response of photosynthesis to UV irradiation. Such species-dependent effects have been reported from a number of studies (Smith et al. 1992; Calkins and Thordardottir 1980). However, photosynthetic activity was more strongly inhibited by UV irradiation in the cyanophyte, *A. flos-aquae*, than in the chlorophyte, *S. capricornutum*. This was unexpected given the presence of mycosporine amino-acid-like UV-screening compounds in the cyanophyte (Garcia-Pichel and Castenholz 1993), although the cellular concentrations of these compounds are known to vary depending on species and growth conditions. In our cultures, *A. flos-aquae* cells were not aggregated into flocs and were therefore probably more susceptible to UV irradiation (W.F. Vincent, Université Laval, Québec, personal communication). In *A. flos-aquae*, inhibition of photosynthesis under UV irradiation was greater at higher photon flux of PAR. This is in contrast with studies on higher plants (Pfündel et al. 1992), where photosynthesis in intact leaves was less affected by UV irradiation applied together with strong PAR intensities.

Under our experimental conditions, the extracellular release of photosynthetically fixed dissolved organic carbon was low in *A. flos-aquae* and *S. capricornutum* during both the light and dark periods. No significant effect of UV irradiation was observed on extracellular release, either immediately after

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Table 3. Effect of UV irradiation on the ratios of dark carbon loss to photosynthesis.

	Low PAR (100 μmol·m^{-2}·s^{-1})		Medium PAR (300 μmol·m^{-2}·s^{-1})		High PAR (550 μmol·m^{-2}·s^{-1})	
	–UV	+UV	–UV	+UV	–UV	+UV
			Aphanizomenon flos-aquae			
4 h dark	0.074	0.065	0.068	–0.031	0.055	0.043
22 h dark	0.019	–0.014	0.017	–0.014	0.014	–0.023
			Selenastrum capricornutum			
4 h dark	0.057	–0.050	0.045	0.017	–0.038	0.070
22 h dark	0.016	0.028	0.018	0.014	0.044	0.013

Note: Rates are calculated as the ratio of Δdpm per hour in darkness to Δdpm per hour in light. Negative values signify that there was a net [^{14}C]bicarbonate uptake in the dark.

the irradiation period or after the subsequent dark incubation, despite the metabolic stress on the algae that might be expected from this treatment.

Respiration

There have been very few studies of the effects of UV radiation on respiration of algae or other plants. Usually the determination of respiration has taken a subservient role to measurements of photosynthesis. For example, Larkum and Wood (1993) who showed significant effects of UV radiation on phytoplanktonic and benthic macroalgal photosynthesis simply noted that they did not observe any effect on respiration and gave no data or the time scale for their respiration measurements.

We determined respiratory activity by three independent methods: dark loss from algal cells of previously incorporated ^{14}C, direct measurement of O_2 uptake, and evaluation of ETS activity. In actuality, these methods reflect different aspects of respiratory metabolism and thus UV irradiation would not necessarily be expected to have identical effects on these measurements.

Dark ^{14}C loss

As noted, extracellular release of dissolved organic carbon in darkness was negligible. It is also possible that some dissolved organic carbon released by the algae during the incubation period may have been taken up by bacteria in these samples and subsequently respired. However, given the very predominant biomass and metabolic activity of algae this would represent a very small carbon flux. Thus, the bulk of the carbon losses from the algal cells that occurred during the dark period must be attributed to respiration. Similar results using this technique were reported by Berman and Kaplan (1984).

Aphanizomenon flos-aquae cultures that were not exposed to UV irradiation exhibited a normal pattern (cf. Berman and Kaplan 1984) of dark ^{14}C loss with a decrease of about 20% of initially incorporated ^{14}C dpm over 22 h (Table 2). At all levels of previous irradiation with PAR, the decrease of cellular ^{14}C was relatively rapid during the first 3 h of darkness (probably because of an EPIR; Falkowski et al. 1985; Beardall et al. 1994) and much slower over the subsequent 20 h. Treatment with UV irradiation during the light phase not only drastically lowered ^{14}C fixation but eliminated the dark drop in cellular ^{14}C. Indeed a slight rise in the cellular ^{14}C counts was observed after 20 h in all treatments. Only in the low-PAR treatment was there a small dark drop in the ^{14}C in cells during the first 3 h.

Selanastrum capricornutum cultures treated with medium or low PAR (no UV) also showed normal patterns of ^{14}C loss. Here the dark carbon loss increased with higher PAR levels (Table 2). Addition of UV irradiation either had no effect (medium PAR level) or accelerated the dark ^{14}C decrease (low PAR treatment). In the case of high PAR (without UV), an initial 3.8-h increase of cellular ^{14}C was observed prior to a relatively rapid ^{14}C loss from 3.8 to 22 h. In this case, treatment with UV and high PAR irradiation decreased respiration rates and stabilized the cellular ^{14}C loss after an initial 3.8-h decline.

In Table 3 we show the ratios of dark carbon loss, i.e., respiration (dpm·h^{-1}) to photosynthetic carbon incorporation (dpm·h^{-1}) as calculated for either the first hours of darkness or the entire 22-h dark period. In almost all cases, the addition of UV irradiance during the light period affected this ratio significantly, although the direction of the effect in *S. capricornutum* was not consistent. Note that all non-UV-irradiated samples showed a loss of ^{14}C dpm during the dark with the exception of the *S. capricornutum* sample taken after 3.8 h.

The continued fixation of ^{14}C in the dark in UV-treated cultures of *A. flos-aquae* and after some treatments in *S. capricornutum* is difficult to interpret. Presumably this could result from an anaplerotic fixation of inorganic carbon to replenish TCA cycle intermediates under conditions of high respiratory activity (Beardall 1989; Li et al. 1993). However, there is little evidence from the data presented here for a correlation between continued significant rates of ^{14}C incorporation in the dark and increased respiration rates as determined by O_2 consumption.

Oxygen consumption

Rates of respiratory O_2 consumption immediately after illumination were directly related to photon flux of PAR in both species tested (Figs. 3 and 4). Rates decreased with time in darkness. This EPIR effect is well documented in microlagae (Gibson 1975; Falkowski et al. 1985; Markager and Sand-Jensen 1989; Markager et al. 1992; Beardall et al. 1994). After 22 h of darkness, rates of O_2 consumption tended to rise in some instances, presumably reflecting changes in cellular metabolism as cells adjusted to prolonged darkness.

In the present experiments with *S. capricornutum*, UV exposure stimulated the oxygen consumption rate when combined with the highest PAR exposure. In contrast, cells of *A. flos-aquae* exposed to high PAR and UV irradiation showed a decrease in oxygen consumption. This is also in keeping with the observation that, in general, dark ^{14}C loss in this organism

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was eliminated in cells that were previously exposed to UV irradiation. These results are consistent with the proposal that EPIR effects are associated with the provision of excess photosynthate during the light, which is subsequently rapidly respired (Markager and Sand-Jensen 1989; Markager et al. 1992; Beardall et al. 1994). Thus, in *A. flos-aquae*, in which photosynthesis was significantly inhibited by UV irradiation, EPIR was also decreased. Conversely, in *S. capricornutum*, in which UV irradiation has little effect on photosynthesis, both UV-treated and nontreated cells showed strong EPIR effects. There was a trend towards generally higher initial oxygen consumption rates in UV-exposed *S. capricornutum*. This would be consistent with the retention of regulated cellular activity after UV exposure being dependent on increased repair processes and maintenance demands.

In conclusion, UV treatment had a negative effect on net carbon accumulation in both species after 2 h of the light exposure, and these effects were still perceptible after a further 22 h in darkness. It is possible to postulate that different mechanisms are involved in the two organisms. In *A. flos-aquae*, carbon fixation rates were inhibited relatively strongly, which caused low accumultion of organic carbon even though subsequent respiration rates were depressed. In *S. capricornutum*, carbon fixation was less affected during PAR + UV exposure. In this case, however, post-irradiation dark respiration rates were enhanced, a process that probably decreased the net fixation of carbon as measured over the 24-h experimental period.

Electron transport system activity

ETS activity per cell tended to decrease in the dark in the eucaryote and increase in the procaryote (Fig. 5). In all cases except *S. capricornutum* subject to high PAR + UV, UV exposure led to an increase in ETS activity immediately upon darkening. However, at the end of the dark period, ETS activities were similar with or without UV pretreatment of cells or were lower for UV-irradiated samples.

In the case of *A. flos-aquae*, ETS activity increased with time in the dark (Figs. 5A and 5B). Cyanobacteria have a unique association between the respiratory and the photosynthetic electron transport systems that share the same cytochrome b_6/f complex (Malkin 1988). This could mean that, in the light, the two systems compete for electrons and the respiratory ETS is repressed, whereas in the dark only the respiratory ETS is operative and its activity increases. In all cases in our study the ETS activity of *A. flos-aquae* was higher at the end of the dark period than initially.

The addition of UV radiation caused a transient ETS increase, at the beginning of the dark period, compared with treatments without UV. This might be related to the strong inhibition of photosynthesis by UV radiation on *A. flos-aquae* during the light period discussed above. If this inhibition acts to partially derepress respiration during the light period, then the observed transient enhancement of ETS activity upon darkening might be expected. The general effect of UV exposure, however, was a decrease of ETS activity, relative to non-UV-treated samples, that was particularly manifested at the end of the dark period in the high PAR + UV *A. flos-aquae* culture. The strong inhibition of photosynthesis that occurred in these cultures (see above) might have resulted in a shortage of photosynthetic products as substrates for respiration during the dark period (Beardall et al. 1994).

Aphanizomenon flos-aquae treated with high PAR without UV irradiation showed the highest ETS activity at the end of the dark period (0.85 nmol $O_2 \cdot 10^6$ cells$^{-1} \cdot$min^{-1}), almost double that obtained with cells treated with high PAR + UV (Fig. 5B). In the absence of UV, prior exposure to high PAR (Fig. 5B) enhanced ETS activity in the dark (probably as a result of higher photosynthesis during the light period). UV irradiation inhibited photosynthesis and, in consequence, respiratory ETS activity in the dark diminished.

We observed respiratory oxygen uptake rates exceeding ETS activity in most cases for *A. flos-aquae* (Fig. 6). The excess O_2 consumption could be due to some process not measured by the ETS assay. The role of the respiratory chain in procaryotic obligate phototrophs is not completely understood. In cyanobacteria, classical inhibitors of the mitochondrial-type cytochrome b/c_1 complex (i.e., antimycin) have little or no effect on oxidoreductase activity of the b_6/f complex. In addition, it has been shown that the respiratory aa_3-type cytochrome *c* oxidase is present in the cytoplasmic membrane of cyanobacteria. When mixed-membrane (i.e., cytoplasmic plus thylakoid) preparations are used to measure respiratory activity, as in our study, the capacity of the enzyme to oxidize reduced cytochrome *c* is lower, possibly because of the diversion of electron flow to other pathways (Fry and Peschek 1988). This would explain the higher oxygen consumption rates (measuring all routes of electron flow to oxygen) than ETS activity (measuring only flux through the mitochondrial-type pathway). Hence, the ETS method is probably not appropriate for measurements of respiratory activity in phototrophic procaryotes.

In the eucaryote *S. capricornutum*, all except one measurement of rates of oxygen uptake were within the capacity of measured ETS capacity (Fig. 6). *Selenastrum capricornutum* showed decreasing ETS values during the dark period. Maximum activity was found upon darkening after treatment with high PAR without UV radiation. This is in agreement with reports of higher ETS activity co-occurring with EPIR in other eucaryotic microalgae after PAR illumination, the magnitude of the enhancement being related to the previous radiation dosage (Martinez 1992). However, high PAR combined with UV radiation transiently inhibited ETS activity in dark. The fact that actual oxygen consumption was enhanced after high PAR + UV suggests that there is some oxygen-consuming process occurring under these conditions that was not measured by the ETS assay. It is possible, for instance, that the mitochondrial cytochrome pathway (CP) was inhibited and the alternate respiratory pathway (AP) was stimulated under these conditions. Weger and Dasgupta (1993) found, in *Chlamydomonas reinhardii*, that AP showed a large increase relative to either CP or total respiration in nutrient-limited versus nutrient-sufficient cells and in cells under temperature or osmotic stress. In the last case, CP was greatly reduced and AP maintained respiratory electron flow. In our study, UV radiation combined with high PAR might cause a similar effect.

It is evident that the effects of UV treatment on respiratory metabolism were not consistent across techniques used to determine respiration (Table 4). This is not unexpected, since each of the approaches measures different aspects of respiratory metabolism. Nevertheless, it may be noted that for *A. flos-aquae*,

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Table 4. Impact of UV irradiation on algal respiration rates measured as oxygen uptake, electron transport system activity, or dark carbon release.

	Low PAR (100 μmol·m^{-2}·s^{-1})			Medium PAR (300 μmol·m^{-2}·s^{-1})			High PAR (550 μmol·m^{-2}·s^{-1})		
	ΔO_2	ETS	Dark C	ΔO_2	ETS	Dark C	ΔO_2	ETS	Dark C
A. flos-aquae	+	–	–(~)	–	nd	–	–	–	–
S. capricornutum	–(~)	–	+	–	nd	0	+	0	–

UV effects on respiration were generally negative (i.e., decreased respiration). The response of *S. capricornutum* was more ambivalent (e.g., contrasting results for ETS and dark carbon loss with the low PAR + UV treatment; Table 4).

Confocal microscopy (data not shown) showed that *S. capricornutum* had far more disruption of cellular integrity following UV exposure than *A. flos-aquae*, which could have been related to the enhancement of respiration after exposure to UV radiation observed in the former organism. Clearly the metabolic processes involved in, and the costs of, repair of UV damage require further investigation.

In conclusion, our experiments indicate that, in some algal species, UV-A + UV-B will affect not only the absolute amounts of photosynthetically fixed carbon and respiration but also the relative activities of photosynthetic and respiratory pathways. The extent of this impact is dependent on species and also on other environmental parameters such as PAR intensity and perhaps on previous nutritional state. Of the two algae in this study, *S. capricornutum* appeared to be relatively insensitive to UV irradiation in terms of photosynthetic and respiratory response whereas both these processes were strongly affected in *A. flos-aquae*. As yet there is little information on the relative impact of UV irradiation combined with high levels of PAR on photosynthesis and other metabolic processes in aquatic organisms (Vincent and Roy 1993), and our present results, although limited, are suggestive.

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Solar ultraviolet radiation dependent decrease of particle light absorption and pigments in lake phytoplankton

Helmut Maske and Mikel Latasa

Abstract: The ultraviolet (UV) spectral component of daylight on a clear day at mid-latitudes can significantly reduce phytoplankton pigments within a daylight period. Phytoplankton samples from Redberry Lake, Saskatchewan, dominated by cyanobacteria were incubated in quartz bottles under optical long band-pass filters (cutoff wavelength 420–305 nm) in daylight in June. After incubation, samples were filtered, and in vivo particle light absorption (380–700 nm) and pigment concentrations were measured. Solar ultraviolet-B irradiance (UV-B; 280–320 nm) was measured radiometrically. On sunny days the samples that were exposed to daylight UV light showed a relative decrease in particle absorption and pigment concentration after the incubation compared with samples exposed only to visible daylight. No such decrease was observed during an overcast day. The UV-B data did not show a clear relation with the degree of pigment reduction, suggesting that other environmental factors or the state of adaptation partially controlled the pigment decrease of phytoplankton under UV-B exposure. Neither specific spectral components of the absorption spectrum nor specific pigments were more easily degraded than others by solar UV light, although the ratio of zeaxanthin to chlorophyll *a* probably increased in cyanobacteria as a result of UV exposure.

Résumé : La composante spectrale ultraviolet (UV) de la lumière par un jour clair, aux latitudes moyennes, peut réduire de façon significative les pigments du phytoplancton au cours d'une période diurne. Des échantillons de phytoplancton du lac Redberry (Saskatchewan) dominés par des cyanobactéries ont été incubés dans des bouteilles de quartz sous filtres optiques passe-bande (longueur d'onde critique de 420 à 305 nm) à la lumière du jour en juin. Après incubation, les échantillons ont été filtrés, et on a mesuré in vivo l'absorption de la lumière par les particules (380–700 nm) et les concentrations de pigments. L'éclairement énergétique solaire par les ultraviolets B (UV-B; 280–320 nm) a été mesuré par radiométrie. Les journées ensoleillées, on observait dans les échantillons exposés à la lumière ultraviolette du jour une diminution relative de l'absorption par les particules et de la concentration de pigments après l'incubation par rapport aux échantillons exposés seulement à la lumière visible. Aucune diminution de ce type n'a été observée pendant une journée où le ciel était couvert. Les données sur les UV-B n'ont pas révélé de relation claire avec le degré de réduction des pigments, ce qui permet de penser que d'autres facteurs environnementaux, ou le degré d'adaptation, régissent en partie la diminution des pigments du phytoplancton exposé aux UV-B. Les UV solaires ne semblaient pas dégrader certaines composantes spécifiques du spectre d'absorption ni certains pigments plutôt que d'autres, mais le rapport de la zéaxanthine à la chlorophylle *a* augmentait probablement chez les cyanobactéries à cause de l'exposition aux UV.
[Traduit par la Rédaction]

Introduction

In recent years the decrease of ozone concentration in the upper atmosphere has resulted in increasing ultraviolet-B radiation (UV-B, 290–320 nm) on the Earth's surface. It has been known for many years that UV-B has detrimental effects on living organisms including phytoplankton. Much research has recently been directed towards a quantitative description of the relationship of primary production and ultraviolet (UV) irradiance in pelagic ecosystems because of the global importance of a potential reduction in primary production in the oceans.

Given the present solar spectral irradiance of UV, the greatest damaging effect to primary production is actually ultraviolet-A irradiance (UV-A, 320–400 nm) (e.g., Maske 1984; Cullen et al. 1992), but UV-B has been the focus of more research because of its tendency to increase (Kerr and McElroy 1993). The understanding of the response of phytoplankton to UV irradiation is still vague because of the many different metabolic components that are affected by UV light. The interpretation is further confounded by several types of UV protective mechanisms in phytoplankton including pigments that may serve as UV umbrellas, repair mechanisms, and components that quench oxygen radicals.

We addressed our experiments at the most basic level of UV impact on the photosynthetic system, namely the reduction of photosynthetic pigments. Pigment reduction can be the combined result of inhibited de novo synthesis and the natural turnover of pigments, or it can also be directly due to photobleaching. So far there is no information available on whether one or both mechanisms are responsible for the UV-induced

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Table 1. Sample origin and experimental conditions.

	Experiment 1	Experiment 2	Experiment 3
	Sample origin		
Date	June 6	June 11	June 13
Time	13:00	10:30	17:00
Depth	15 m	10 m	1 m
	Experimental conditions		
Concentrating the sample with 22-μm net	10.5 L concentrated into 1.5 L	20 L concentrated into 1.5 L	
Temperature control during exposure	Lake water, 16.8°C, and tap water, 15°C	Lake water	Tap water, 15°C
Sample containment and exposure period	15:00 to 18:00 daylight at lake 18:00 to 11:00 dark, 16°C; 11:00 > 15:00 daylight at laboratory	11:00 to 15:30 daylight at lake	17:00 to 09:40 dark, 15°C; 09:40 to 17:00 daylight at laboratory
Average UV-B irradiance ($J \cdot s^{-1} \cdot m^{-2}$)	2.7	2.6	3.5
UV-B exposure ($kJ \cdot m^{-2}$)	70	50	93

Note: All samples were prescreened with a 250-μm net.

reduction in pigments. Little research has been directed towards the effect of UV irradiation on photosynthetically active pigments despite the obvious importance for light-limited photosynthesis. Nevertheless, in a recently published list of physiological mechanisms that might explain UV-induced photoinhibition (Smith et al. 1992), pigment reduction was not included.

Plant pigments can be bleached by high irradiance of visible light or long-wavelength UV-A as has been documented for higher plants and for phytoplankton. This decrease could be considered part of the acclimatization process to higher irradiance levels or it could be considered photodamage as there are no strict criteria to distinguish between the two responses. Yentsch (1974) showed that a 1-h exposure to bright daylight in the Sargasso Sea can rapidly decrease chlorophyll concentrations of marine phytoplankton and that the relative decrease depends on the state of adaptation: the surface sample chlorophyll concentration decreased to 48% of the initial value and in the 75-m sample chlorophyll decreased to 26% of the initial value (these experiments were probably performed in glass bottles that excluded part of UV-B). Cullen and Neale (1993) reported a decrease in chlorophyll concentrations of tropical Pacific phytoplankton to about 30% of the initial value when surface sea water was stored in a polycarbonate carboy (cutoff wavelength 365 nm) in sunlight during 75% of a daylight period. This sample was probably not high-light acclimated because the water column was mixed to >50 m depth. From the above reports it is clear that the visible and UV-A spectral components of daylight can reduce the pigment concentration of phytoplankton significantly even without complete daylight UV-B at exposure periods of less than one daylight period.

Few experimental data have been published that concern specifically UV-dependent degradation of phytoplankton pigments, including UV-B. Gessner and Diehl (1951) investigated the UV-induced phytoplankton pigment decrease in freshwater phytoplankton and cultures (*Chlorella, Scenedesmus, Ankistrodesmus*). On clear, sunny days the chlorophyll concentration decreased significantly more in phytoplankton exposed to the complete daylight spectrum than in controls protected by glass sheets with a cutoff wavelength of approximately 350 nm. Steemann Nielsen (1964) reported a decrease in chlorophyll concentration after exposure to full daylight. El-Sayed et al. (1990) found in natural Antarctic phytoplankton that pigment concentrations decreased owing to the UV component in natural daylight within 24 or 48 h. Helbling et al. (1993) showed that daylight UV-A and UV-B at latitude 33°S during spring could decrease the chlorophyll concentration in cultures of *Nannochloris oculata* in addition to the decrease induced by visible light. Cultures of *Isochrysis galbana* showed no corresponding decrease. According to Goes et al. (1994) the carotinoid concentration increased and the chlorophyll concentration decreased significantly in *Tetraselmis* cultures when artificial UV-B was added to visible light. Lesser et al. (1994) found that the chlorophyll concentration in *Thalassiosira pseudonana* cultures showed a tendency to decrease in artificial visible light if UV light was added.

Given the recently observed increase of UV-B in Canada (Kerr and McElroy 1993) and the potential importance of UV photoinhibition to aquatic primary production, we measured the UV-dependent decrease of photosynthetic pigments in lake phytoplankton. We also measured particle absorption and compared it with the pigment decrease, thus taking account of a possible change in the optical geometry of the phytoplankton.

Materials and methods

Samples were taken from the center of Redberry Lake (52°43′N, 107°09′W), a 17 m deep, saline, oligotrophic prairie lake (Table 1; Robarts et al. 1992). For experiments 1 and 2 the samples were screened with gauze (25-μm mesh size) with the intention of concentrating the phytoplankton. Because of the small phytoplankton cell size the only negligibly higher concentrations were found in our concentrated sample. Samples were incubated in 120-mL quartz bottles with Teflon stoppers. The incubator had separate wells for each sample bottle. On top of the wells were mounted long-pass cutoff filters (type KF, Schott) or neutral density filters (metal screens). The long-pass filters cut off different portions of the short-wavelength UV spectrum up to the designated wavelength. The longer wavelength UV and PAR (photosynthetically active radiation; 400–700 nm) were transmitted with approximately 5% loss. The neutral-density filters were checked for their transmittance of PAR, UV-A,

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and UV-B radiation with field radiometers with cosine sensors against daylight. The 20% transmission neutral density (TND) filter was confirmed to transmit 20% in these three spectral regions. The 6% TND filter transmitted 6% PAR and UV-A but no measurable UV-B.

During the sample exposure the incubator was pointed towards the sun in such a fashion that the sun was perpendicular to the surface of the optical filter and the sample bottles were not shadowed. Because of the design of the incubator the access of diffuse sky light to the sample was only about half of the total sky light, thus effectively reducing the downwelling scalar surface irradiance received by the samples.

The measured reflectance of the wet gray PVC material of the incubator was approximately 3% in the UV-B region and 12% in the visible part of daylight. For the reflectance measurement a dry polytetrafluorethylen (PTFE) plate was used as a 100% reference. The reflectance of the incubator material will increase the effective irradiance exposure of the samples proportionately and will partially balance the reduced irradiance resulting from restricted access of diffuse sky light to the incubation wells. For a rough estimate consider that for visible light the contribution of the diffuse sky light on a clear day is approximately 10% of downwelling scalar irradiance in air. If only half of the sky light can enter the sample wells (5%) then the irradiance exposure will be 6% higher ($(1 - 0.05) + (1 - 0.05) \times 0.12 = 1.06$) than for an ideal sample exposed to daylight in a bottle placed on a black horizontal surface. The contribution of diffuse sky light UV-B to scalar downwelling UV-B is approximately 50% on a clear day (data not shown). An analog calculation for UV-B ($(1 - 0.25) + (1 - 0.25) \times 0.03 = 0.77$) shows that UV-B exposure in the incubator is 23% less than for the ideal sample.

Approximately every hour, samples were mixed by gently turning the bottles. Several times during the exposure period the stoppers were opened to allow for air exchange. The sample bottles were half immersed in water flowing through the incubator to maintain a constant temperature.

Downwelling UV-B irradiance (cosine response) was measured with a Brewer ozone spectrophotometer with high spectral (290–320 nm, 0.5 nm) and temporal resolution (approximately 0.25 h) during the time of the experiments. The monitoring site was 76 km from the site of incubation in experiment 2 and during half of the incubation period in experiment 1 and 6 km from the site for the rest of the incubations. We considered the general weather reported at the two locations to be sufficiently similar to justify the use of these UV-B data, but the data can only be considered approximate irradiances for our experiments.

Particle absorption measurements were made by the filter pad method (Trueper and Yentsch 1967) with path-length correction (cf. Mitchell 1990). We used a single batch of Whatman GF/F filters for the calibration of the method with phytoplankton cultures and for the samples. Samples were kept cold (near 0°C) and in the dark in quartz bottles until vacuum filtration at less than 20% atmospheric differential pressure shortly before the absorption measurement in a double-beam spectrophotometer (Pye-Unicam 8-400 UV/VIS). A wetted filter was used as reference. The sequence of sample processing was chosen to follow a pattern unrelated to the wavelength of the cutoff filters. For experiments 2 and 3, duplicate samples were filtered before the daylight exposure (t_0) and kept frozen until the absorption of the filters was measured together with the other samples after the daylight exposure. For the calibration of the filter-pad method the path-length amplification factor was determined from suspensions of algal cultures (*Clamydomonas* sp., *Aphanezomenon* sp., *Synechococcus* sp., and *Chlorella* sp.). Relatively small species were chosen so that they would have sizes similar to those of the lake phytoplankton (see below).

The culture suspensions were diluted to different optical densities and their absorbance was measured in a round cuvette of 1-cm path length and 2 cm diameter using distilled water as a blank. The cuvettes were mounted directly before the entrance of the detector with a diffusor (paper) placed between the sample and the detector entrance. The volume particle light absorption of the samples was calculated from the measured optical density (OD_λ) by subtraction of the OD at 750 nm, applying the experimentally derived linear model of the path-length amplification factor (β), dividing by the virtual sample path (l; m) and changing the OD to the absorption coefficient (a; m^{-1}): $a = 2.3\beta(OD_\lambda - OD_{750})/l$. The virtual sample path length is calculated from the effective filter area (πr^2) and the volume filtered (V; m^3): $l = V/(\pi r^2)$. The particulate absorption coefficients of the samples were not corrected for detrital absorption.

The coefficient of variation of the sample treatment and the particle absorption measurement can be estimated using duplicate samples if normal distribution is assumed. In our experiments we duplicated the total UV-R samples and the t_0 samples. If x_1 and x_2 are the duplicate samples, the mean (μ) is 1 and the 80% confidence limit interval ($\mu \pm 1.28$ coefficient of variation) can be estimated from $X_1 - (X_2 - X_1) < \mu < X_2 + (X_2 - X_1)$, where $X_1 = 2x_1/(x_1 + x_2)$ and $X_2 = 2x_2/(x_1 + x_2)$ (Sachs 1978). From the average confidence interval obtained from various duplicates the coefficient of variation can be estimated by dividing the 80% confidence interval by 1.28. For the particle absorption method we used four sample pairs at 675 and 440 nm and one pair at 350 nm. We calculated from these nine duplicates a coefficient of variation of 16%. For the pigment determination, including the sample manipulation, we estimated from three zeaxanthin, three chlorophyll *a*, and two beta carotene pairs a coefficient of variation of 26%.

After the particle absorption measurements the samples were frozen and transported to the high-performance liquid chromatography (HPLC) laboratory. Samples were extracted in 90% acetone overnight after sonication in an ice-cold water bath for 10 min. The method by Wright et al. (1991) with slight modification was used as the HPLC pigment separation protocol. Pigment concentrations were quantified using response factors obtained from pure standards. Chromatographic peaks were identified by their retention times.

Results

The filter-pad method was calibrated by comparing the absorption of filtered phytoplankton culture with the absorption of phytoplankton suspensions. The data in Fig. 1 are comprised of 13 different culture suspensions measured at 10 wavelengths. The comparison yielded two distinct patterns: *Chlamydomonas* sp. and *Synchechococcus* sp. showed a linear behavior, and therefore the amplification factor (β) of the filter-pad method was a constant. *Aphanizomenon* sp. and *Chlorella* sp. yielded the commonly observed hyperbolic pattern (Mitchell 1990), i.e., β depended on the OD of the filtered sample (Fig. 1). These latter cultures showed a behavior (Fig. 1) that could be well described by the path-length amplification correction recently published by Cleveland and Weidemann (1993). There is no theoretical basis to model the path-length amplification factor; therefore, there is no a priori preference to a certain behavior of the amplification factor. We choose β to be a constant calculated by linear regression with forced intercept of the origin. The true OD of a suspension of cells can then be calculated from the filter-pad data: $OD_{suspension} = OD_{filter\ pad}/2.05$. The hyperbolic correction curve would not have altered our results substantially because our sample OD values were in the range where both linear and hyperbolic correction were similar.

The particle in vivo absorption spectra of the lake samples showed a pronounced shoulder or peak at approximately 565 nm (data not shown), which should be due to phycoerythrin absorption. Although we did not observe our samples

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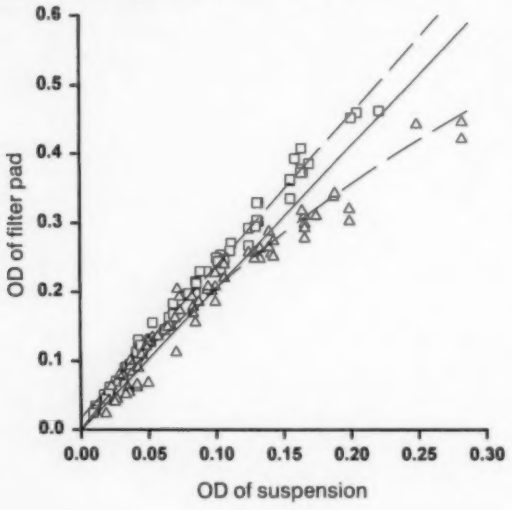
Fig. 1. Calibration of the filter-pad particle absorption method. Squares are *Chlamydomonas* sp. and *Synchechococcus* sp., and triangles are *Aphanizomenon* sp. and *Chlorella* sp. The path-length amplification factor for the squares is described by a straight line with an intercept at the ordinate (broken line). The triangles follow an exponential curve (Cleveland and Weidemann 1993) (broken line). The solid straight line that passes through the origin was used to calculate particle absorption from our filter-pad data (all data: $OD_{filter} = 2.05(OD_{suspension})$, $n = 128$, $r^2 = 0.89$).



under the microscope we conclude from the phycoerythrin peak that the lake phytoplankton was dominated by cyanobacteria. Also, the small size of the phytoplankton suggests that a large fraction were coccoidal cyanobacteria.

The measured UV-B irradiance during the three experiments decreased five orders of magnitude between 320 and 290 nm (data not shown); therefore, the irradiance below 290 nm was considered negligible and the samples that received total daylight were treated like samples under 290-nm cutoff filters in the figures and in the data analysis.

Experiment 1

The lake water column was stratified and chlorophyll concentrations increased towards the bottom. The sample was taken at 15 m depth (cf. Table 1) at a maximum chlorophyll *a* concentration of 2.50 mg $C \cdot m^{-3}$ and pheopigment concentration of 1.2 mg $C \cdot m^{-3}$ (data not shown). The pigment composition of the lake phytoplankton consisted mainly of chlorophyll *a*, zeaxanthin, and carotenes. Xanthophylls showed negligible concentrations. This pigment signature is typical of cyanobacteria. The small size of the coccoidal cyanobacteria can explain our failure to concentrate the lake sample with a 22-μm gauze and the almost identical pigment composition of the screened and unscreened samples.

The weather during sample exposure was variable, with an average PAR irradiance of 900 $\mu E \cdot m^{-2} \cdot s^{-1}$ (1 $\mu E \cdot m^{-2} \cdot s^{-1}$ = 0.217 $W \cdot m^{-2}$) during the incubation time. After the exposure the particle absorption of those samples exposed to short-wavelength UV-R had decreased 25% at 440 nm and 60% at the 675-nm chlorophyll peak (Fig. 2A). The particle absorption at 675 nm decreased monotonically with shorter wavelengths of exposure whereas the 440-nm sample decreased with exposure between 400 and 350 nm. The 20 and 6% TND filters increased the particle absorption at 675 nm from 15% (20% TND filter) to 40% (6% TND filter) but the 440-nm particle absorption increased only 20% (6% TND filter).

The chlorophyll concentration (Fig. 3A) generally followed the pattern of particle absorption. The concentration decreased monotonically with exposure from 420 nm towards shorter wavelengths. The sample exposed to full daylight decreased its chlorophyll *a* concentration 60%. The decrease of pigments and the absorption at 675 nm were significantly related to the cutoff wavelength (Table 2). The TND filters increased the chlorophyll *a* concentration by 35% (20% TND filter) and 50% (6% TND filter). The zeaxanthin concentration decreased by 40% in the full-exposure sample and increased insignificantly with the neutral-density filters.

Experiment 2

For details see Table 1. The weather was overcast with an average PAR irradiance of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ during the incubation time. The samples incubated at different wavelengths all showed, with one exception, higher particle absorption than the sample incubated under a 420-nm cutoff filter. There was no discernible trend of particle absorption with wavelength of solar UV exposure (Fig. 2B, Table 2). There was no change in particle absorption in the 20% TND filter sample, but the particle absorption in the 6% TND filter sample increased by 40% at 440 nm and 70% at 675 nm. Pigment concentration in all samples did decrease significantly during the daylight exposure if compared with the t_0 concentration (Fig. 3B). The chlorophyll *a* concentration followed an irregular pattern with exposure to daylight, with a general tendency to decrease with exposure under shorter wavelength cutoff filters (Table 2). The samples under TND filters showed increased (20% TND filter) or unchanged chlorophyll *a* concentrations (6% TND filter). The zeaxanthin showed no significant decrease with cutoff wavelength (Fig. 3B, Table 2).

Experiment 3

The weather was clear during the incubation period. The particle absorption (Fig. 2C) showed a general decrease with exposure to shorter UV wavelengths. The sample exposed to total solar UV light had approximately 30% less particle absorption at 675 nm than the sample covered with the 420-nm cutoff filter. The major part of this decrease took place in the spectral range between 420 and 350 nm; for that reason the rank correlation of the absorption at 440 nm versus cutoff wavelength showed low statistical significance (Table 2). Incubation under the 20% neutral-density screen yielded a result similar to that under the 420-nm cutoff filter but incubation under the 6% TND filter increased absorption by 10%. The pigment concentration before the daylight exposure was about 5% higher at 675 nm and 20% lower at 440 nm than that of the 420-nm sample.

The pigment concentration (Fig. 3C) showed the same general pattern as particle absorption. Chlorophyll *a* concentration decreased 40% in the sample exposed to full UV radiation compared with the 420-nm sample, it increased 30% in the sample under the 6% TND filter. Zeaxanthin concentrations (Fig. 3C) behaved similarly to chlorophyll *a*

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Table 2. Nonparametric correlation (R_s, Spearman rank order correlation) of in vivo absorption and pigment concentration versus cutoff wavelength during exposure to daylight, using the data between total UV and 420 nm (the samples exposed to total UV were set to 290 nm).

Experiment	Pigment	R_s	p	In vivo absorption (nm)	R_s	p
1	Chlorophyll *a*	0.997	<0.001	675	0.948	<0.001
1	Zeaxanthin	0.948	<0.001	440	0.547	0.102
2	Chlorophyll *a*	0.753	0.012	675	–0.419	0.228
2	Zeaxanthin	0.444	0.199	440	–0.547	0.102
3	Chlorophyll *a*	0.839	0.002	675	0.748	0.013
3	Zeaxanthin	0.747	0.013	440	0.477	0.194

Note: In all tests $n = 10$, except for experiment 3, where $n = 9$.

Fig. 2. Relative in vivo particle light absorption after daylight exposure versus cutoff wavelength in (A) experiment 1, (B) experiment 2, and (C) experiment 3. Data are normalized to the 420-nm sample. The samples receiving the complete UV spectrum (without cutoff filters) were set at 290 nm on the abscissa. Squares are particle absorption at 675 nm, and triangles are particle absorption at 440 nm.

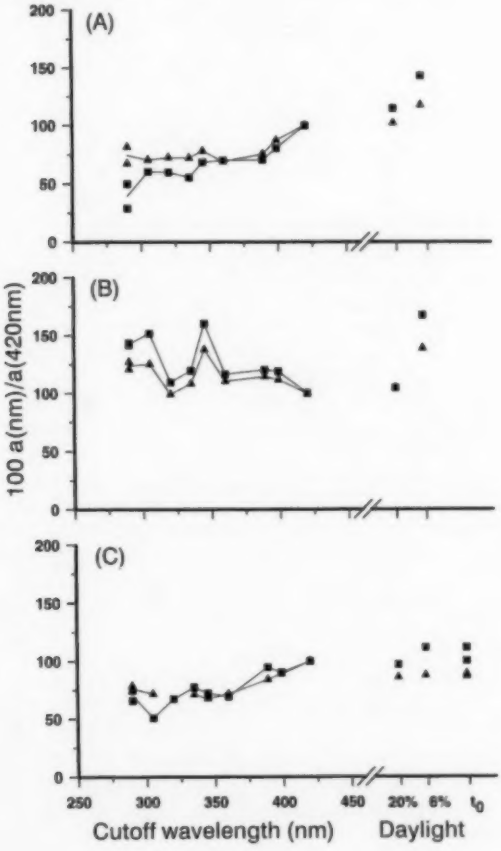
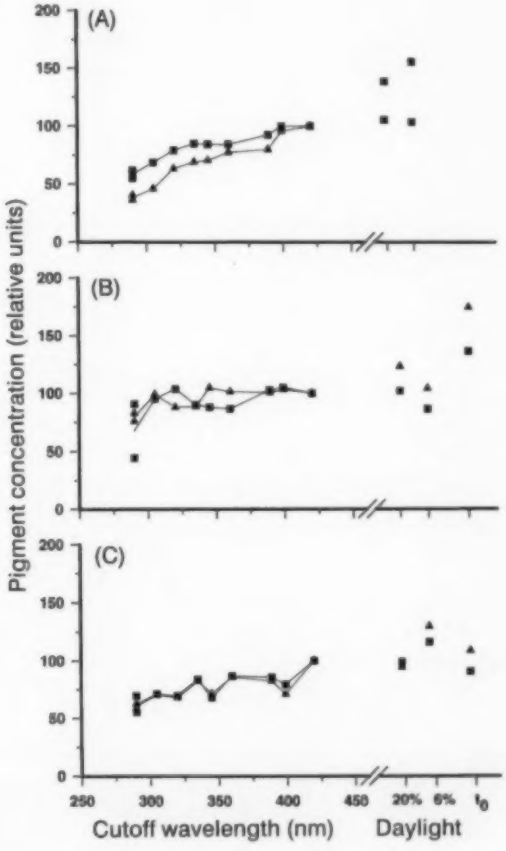


Fig. 3. Relative pigment concentration after daylight exposure versus cutoff wavelength in (A) experiment 1, (B) experiment 2, and (C) experiment 3. Data are normalized to the 420-nm sample. The samples receiving the complete UV spectrum (without cutoff filters) were set at 290 nm on the abscissa. Triangles are chlorophyll *a* concentration, and squares are zeaxanthin concentration.



concentrations but did not show the same increase as chlorophyll *a* with the 6% TND filter. Both pigment concentrations showed a significant relationship with the cutoff wavelength (Table 2).

Discussion

In two of three experiments absorption and pigment concentrations decreased significantly owing to exposure of the ultraviolet

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Fig. 4. Specific absorption coefficients of chlorophyll *a* at 675 nm after daylight exposure versus cutoff wavelength. Circles are experiment 1, squares are experiment 2, and triangles are experiment 3.

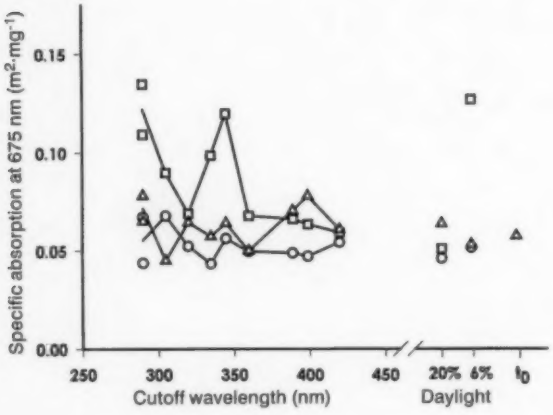
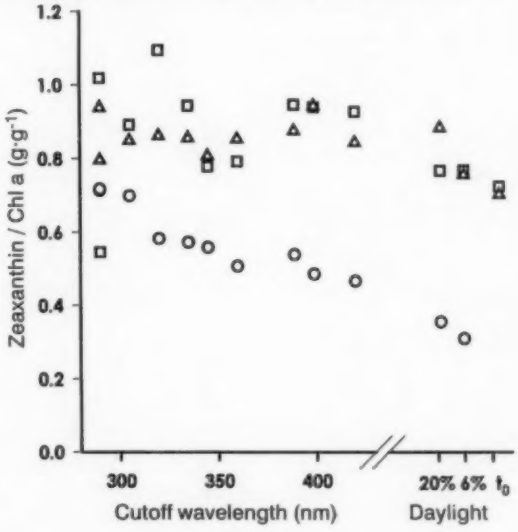


Fig. 5. Pigment ratio of zeaxanthin/chlorophyll *a* versus cutoff wavelength during daylight exposure. Circles are experiment 1, squares are experiment 2, and triangles are experiment 3.



spectral component of daylight (Figs. 2 and 3). The decrease was between 25 and 50% after exposure periods of less than 1 day in comparison to samples that were exposed only to the visible daylight. The natural phytoplankton in our samples was mostly composed of coccoidal cyanobacteria. Gessner and Diehl (1951), with a similar experimental design and at similar latitudes, reported a chlorophyll decrease of about 44% in phytoplankton cultures and in natural freshwater phytoplankton samples. When they looked at the natural samples after the UV exposure under the microscope they found no significant difference in sensitivity between groups of phytoplankton.

Particle light absorption data showed no indication that any part of the absorption spectrum was particularly sensitive to UV exposure (Fig. 2). However, in experiment 1 the absorption peak in the blue range showed a smaller decrease in absorption after UV exposure (Fig. 2A). In this experiment, zeaxanthin, which contributes to the absorption in the blue but not in the red range, also underwent a smaller decrease than chlorophyll *a* concentration, which is the only pigment found in the samples that absorbed in the red range. The difference in pigment reduction under UV exposure could explain in part the relatively smaller absorption decrease in the blue than in the red range. Also, because we did not correct for detrital absorption, the contribution of the detritus to the particle absorption in the blue range has to be taken into account. Because detritus probably decreased little after exposure to UV, the particle absorption in the blue range was expected to decrease proportionately less than particle absorption in the red range, even if the particle absorption decreased proportionately. The samples for the first experiment were taken close to the lake floor, and therefore they probably contained a higher portion of resuspended detritus. The difference in the responses of the particle absorption at 440 and 675 nm was particularly evident in those samples (Fig. 2A).

The specific absorption coefficient of chlorophyll at 675 nm showed no significant change with different spectral UV exposure (Fig. 4) except in experiment 2 where the results seem to behave in an erratic way. The relative constancy of specific absorption suggests that UV radiation did not affect the arrangement of chlorophyll within the cells or did not selectively reduce the chlorophyll concentration of certain cell sizes. The average values of specific absorption (Fig. 4) are considerably higher than expected at 675 nm (cf. Bricaud et al. 1995). A likely explanation is that the pigment concentrations were underestimated because the solvent used for extraction (90% acetone) only partially extracted the samples. Another possible explanation is that the chlorophyll on the sample filters degraded during the absorption measurement (Stramski 1990) and when the same filters were subsequently extracted the pigments were underestimated. The relative constancy of the specific absorption coefficient in experiments 1 and 3 indicates that the mechanism that led to the underestimation of chlorophyll had a relatively constant effect.

The pigment ratio (zeaxanthin/chlorophyll *a*) stayed relatively constant in the different samples of experiments 2 and 3 (Fig. 5). In experiment 1 the pigment ratio increased with shorter wavelength UV exposure. A likely explanation for this increase is photoacclimatization, where zeaxanthin serves as a photoprotective pigment that is increasingly synthesized for cell protection with more UV exposure. The sample for experiment 1 was low light adapted because it came from the lake bottom underneath a stratified water column. Its low zeaxanthin concentration in the samples of experiment 1 under TND filters and without UV exposure should be indicative of the original state of the sample; unfortunately, for this experiment there are no pre-exposure data available. The pattern of increasing zeaxanthin/chlorophyll *a* ratio with increasing UV exposure in cyanobacteria is similar to the pattern observed by Quesada et al. (1995) in Antarctic cyanobacteria. They estimated total carotenoids from the in vivo absorption at 490 nm and found variation in the ratio from 1.15 to 1.35 (relative units). This relatively small variation compared with our experiment 1 could be explained by the in vivo absorption of other pigments at 490 nm that do not respond to the UV exposure.

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Zeaxanthin is generally considered a photoprotective carotinoid that is formed in higher plants by de-epoxidation of violaxanthin (Demming-Adams and Adams 1992). Cyanobacteria do not have violaxanthin, and therefore the zeaxanthin concentration has to be regulated by de novo synthesis and degradation. In higher plants zeaxanthin formation can be induced by high irradiance of visible light and is not specific to UV whereas in our experiment 1 the zeaxanthin/chlorophyll *a* ratio appeared to be related to UV exposure.

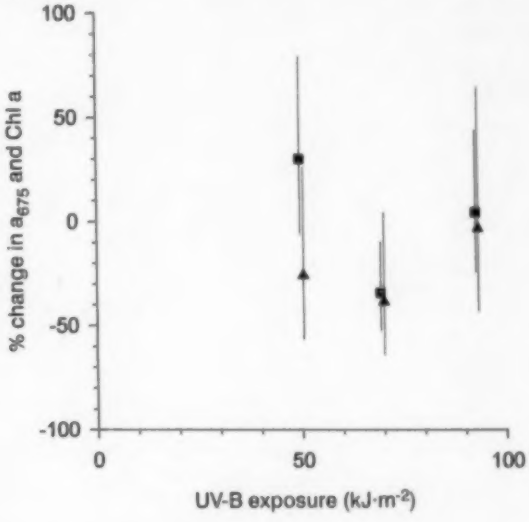
UV-B exposure was different in the experiments mainly because of the different lengths of exposure periods and not because of differences in cloud cover (cf. average UV-B irradiance in Table 1). Nevertheless, when the differences in absorption coefficient and in pigment concentration between samples exposed to full daylight and samples exposed only to irradiance above 320 nm are plotted against UV-B exposure (integral over time of downwelling irradiance, 280–320 nm), no significant trend was found (Fig. 6). This comparison suffers somewhat from the different geometries of the UV-B measurement, with a cosine response, and the exposure of the samples, which had an approximately 2π geometry. As can be seen from the data in experiments 1 (Figs. 2A and 3A) and 3 (Figs. 2C and 3C) a major part of the decrease in absorption and pigments was induced by irradiance above 320 nm and not by UV-B. The detrimental effect of UV-A is similar to the photoinhibition of photosynthesis in middle latitudes (Maske 1984). Also, the magnitude was similar, although as mentioned above it is unlikely that the pigment decrease is responsible for the photoinhibition of photosynthesis under saturating irradiances. The mechanism of pigment reduction is also unclear, but it is unlikely that the high-energy photons of UV-B can effectively bleach the pigments because low irradiances of UV-B can support photosynthesis (Halldal 1967).

The pigment decrease observed in our experiments did not result in degradation products with absorption in the visible spectrum (350–720 nm), i.e., no shift in peak wavelength was observed, neither did the HPLC chromatograms show new peaks. This follows the results of Nelson and Robertson (1993) who exposed killed phytoplankton cultures to visible light and found that the absorption spectrum (250–700 nm) decreased during exposure in a way that suggested stable detrital absorption and a strong decrease in photosynthetic pigments. From their absorption spectra no new peaks are apparent after exposure.

Conclusions

Our data show that pigments from cyanobacteria are sensitive to the UV spectral component of sunlight. At mid-latitudes, during one daylight period the UV exposure reduced the concentration of photosynthetically active pigments to about half that of samples exposed only to the visible part of daylight. In a stratified water body under clear sky conditions the concentration of photosynthetic pigments that should typically increase during the day owing to de novo synthesis in light will therefore decrease near the water surface. The reduced pigment concentration will lower potential photosynthesis at low light levels. It is unlikely that the UV-dependent pigment reduction is responsible for the UV-dependent photoinhibition of carbon uptake (Maske 1984; Smith et al. 1992), because under those experimental conditions PAR should not be limiting even if the absorption cross section is reduced to half. Only when the surface phytoplankton population is mixed to greater depth or at greater solar zenith angles will the pigment reduction result in reduced photosynthesis. It should be investigated if the UV-dependent pigment decrease is part of the physiological adjustment to high PAR levels over time scales of hours and therefore beneficial to the phytoplankton. The decrease of pigments near the water surface could be of some importance to the interpretation of remotely sensed ocean color, because ocean color images are taken only during clear sky conditions when UV-dependent pigment reduction is expected.

Fig. 6. Relative changes in the particle absorption at 665 nm (a_{675}) and in chlorophyll *a* concentration of the total UV sample (S_{UV}) relative to the 320-nm (S_{320}) sample, versus UV-B exposure. Squares are the in vivo particle absorption at 665 nm, and triangles are the chlorophyll *a* concentration. The error bars are calculated with the coefficient of variation (CV) of each method. The upper estimate was calculated as $(S_{UV}(1 + CV/100))/(S_{320}(1 - CV/100))$; the lower estimate was calculated as $(S_{UV}(1 - CV/100))/(S_{320}(1 + CV/100))$.



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Physiological responses of natural plankton communities to ultraviolet-B radiation in Redberry Lake (Saskatchewan, Canada)

Gustavo A. Ferreyra, Serge Demers, Paul del Giorgio, and Jean-Pierre Chanut

Abstract: Damaging effects of ultraviolet radiation on the aquatic biota, related to anthropogenic modifications in the ozone layer, have been extensively described. However, most of the research has focused on marine environments, and information about the effects of ultraviolet radiation on saline prairie lakes of Canada is lacking. To test the deleterious effects of ultraviolet-B (UV-B) radiation on the planktonic community, two exposure experiments were performed in Redberry Lake, Saskatchewan. The responses of primary productivity, phytoplankton chlorophyll *a* and size, bacterial changes, and the electron transport system to natural UV-B fluxes reaching the surface of the lake were studied. No clear effects of UV-B on phytoplankton carbon assimilation and chlorophyll *a* were observed. However, significant responses were found for the two phytoplankton size fractions studied (0.7–2 and >2 μm), which were more related to the experimental conditions than to UV-B effects. Bacteria presented a clear decrease in cell number under the highest UV-B doses, whereas the opposite was observed for the electron transport system activity.

Résumé : Les effets des radiations ultraviolets (RUV) sur les écosystèmes aquatiques ont fait l'objet de nombreuses études au cours des dernières années. Cependant, la plupart des travaux ont touché les environnements marins et l'information concernant les effets des RUV sur les lacs salés des prairies canadiennes est à peu près inexistante. Dans le but de déterminer les effets potentiels des RUV sur les communautés planctoniques de ces types de lac, deux séries expérimentales ont été effectuées dans le Lac Redberry (Saskatchewan). Les réponses au niveau de la production primaire, de la taille et la chlorophylle *a* phytoplanctonique, de la concentration bactérienne et de l'activité du système de transport des électrons (electron transport system) ont été étudiées sous des conditions naturelles de RUV de type B (RUV-B). Des effets peu clairs des RUV-B sur la production primaire et la biomasse du phytoplancton ont été observés. Cependant, des différences significatives ont été observées entre les deux fractions du phytoplancton étudiées (0,7–2 et >2 μm), lesquelles semblent avoir été plus reliées aux conditions imposées dans les experiences qu'aux effets des RUV-B. Le nombre de bactéries montre une diminution marquée dans les contenants soumis aux RUV-B tandis que l'activité du système de transport des électrons présente plutôt une augmentation dans ces mêmes contenants.

Introduction

Ozone depletion has been described to be significant in some locations of the earth (Crutzen 1992), including the Northern Hemisphere (Hofmann and Deshler 1991; Madronich 1992). The increase of anthropogenic gases in the atmosphere is thought to be responsible for the decrease in thickness of the stratospheric ozone layer, which absorbs ultraviolet-B radiation (UV-B; 280–320 nm). The UV-B wave band represents a small percentage of the total energy reaching the surface of the earth, but is responsible for significant photochemical effects on the aquatic environment (Frederick and Lubin 1988). Biologically effective levels of solar ultraviolet radiation (UVR) penetrate the water column to significant depths (Jerlov 1950). For example, in clear lake and ocean environments UV-B penetrates the water column down to 60 m (Smith and Baker 1981; Smith et al. 1992; Vincent and Roy 1993). In highly productive coastal regions of Antarctica biologically significant levels of UVR have been observed to 30 m depth (Karentz and Lutze 1990).

A rapidly increasing number of studies suggest that UV-B radiation is harmful to aquatic organisms like bacterioplankton (Thomson et al. 1980; Mohering 1980; Sieracki and Sieburth 1986; Herndl et al. 1993) and phytoplankton (reducing their productivity) (Steemann Nielsen 1964; Jitts et al. 1976; Smith et al. 1980; Cullen et al. 1992). Vincent and Roy (1993)

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reviewed the major effects of UVR on aquatic and marine environments. Several studies show a decline in the primary production rate (Smith 1989; Cullen and Lesser 1991; Häder and Worrest 1991) and a change in phytoplankton community composition (Calkins and Thordardottir 1980; Worrest 1983; Smith et al. 1992; Helbing et al. 1992). Long-term experiments (1–2 weeks) have shown that UVR can reduce cell division rates (Calkins and Thordardottir 1980), induce DNA damage (Karentz et al. 1991), and alter species composition and algal standing crop (Gessner and Diehl 1951; Worrest et al. 1981*a*, 1981*b*; Jokiel and York 1984). In addition, the significance of the effects of UVR at different trophic levels of the community was recently highlighted by Bothwell et al. (1993, 1994).

Most of the prairie lakes of Canada are saline (Hammer 1990). This includes the lakes of southern Sakatchewan, a region where the high degree of lake salinity is of concern in terms of its effects as a limiting factor for fisheries (Rawson and Moore 1944). Information about UV-B effects on aquatic organisms inhabiting these environments is lacking. To study the responses to UV-B at the plankton community level, a research study was conducted in June 1993 at Redberry Lake, Saskatchewan. This lake has been described as having a Z_{max} of 17 m and a mean Z_{eu} of 15.2 m, which means that phytoplankton should be within the euphotic zone most of the time (Robarts et al. 1992). The presence of a marked thermocline in which water temperature drops from approximately 15 to 2°C at around 4–6 m depth, however, can reduce mixing between the epilimnion and the hypolimnion. This could lead to the separation of phytoplankton into two populations adapted differently to light conditions, as suggested by Robarts et al. (1992). One of them, the hypolimnetic population, is shade adapted and the other, the epilimnetic one, is high light adapted. The goal of the experiment was to study the temporal (1–3 days) and vertical (4 m depth, light-adapted community; 16 m depth, shade-adapted community) effects of environmental UVR on the primary productivity and chlorophyll *a* concentration of two phytoplankton size fractions from natural planktonic communities, as well as on bacterial changes and respiration of the microbial community.

Materials and methods

Experimental design

Experimental fieldwork was carried out from June 9 to June 14, 1993, at Redberry Lake (52°43′N, 107°09′W), a south-central Saskatchewan saline lake. Dominant features of this water body are the existence of a marked thermocline during the ice-free period, a high concentration of total dissolved solids (TDS) and dissolved organic carbon (DOC), the presence of sulfate as the most important anion, and oligotrophic conditions (Robarts et al. 1992; Tumber et al. 1993).

Water sampling was carried out at a deep station (16 m) located in the central area of the lake. Forty-litre water samples were collected from 4 and 16 m depths (epilimnion and hypolimnion, respectively) by using opaque Niskin bottles. The water from each depth was filtered through a Nitex net (30-µm mesh) to avoid the presence of large grazers, divided into two subsamples of 20 L, and placed in four plastic containers immersed in a large tank. This system was incubated outdoors. Water in the containers was maintained at ambient temperature (around 16°C) by continuous pumping of surface water into the tank. One subsample from each depth was exposed to the full spectrum of natural light (+UV-B containers), whereas the two other subsamples were partially UV filtered with a glass cover (–UV-B containers). The spectral distribution of light transmission of this filter is shown in Fig. 1, as measured with a Perkin-Elmer Lambda Series spectrophotometer. The glass cover screened out around 10–20% of the photosynthetically active radiation (PAR; 400–700 nm), 40% of the ultraviolet-A (UV-A, 320–400 nm) and 99% of the UV-B incident radiation. We assumed that photoinhibition by PAR was similar in the two containers, with and without glass cover, and that the difference in PAR between containers was negligible when compared with the difference in UV-B under the two experimental conditions.

Two experiments were performed. During the first experiment the subsamples from 4 and 16 m were incubated as described for 2 and 3 days, respectively (from June 10 to June 12 for 4 m and from June 9 to June 12 for 16 m). During the second experiment a new set of water subsamples from both depths was incubated for 1 day (June 13–14).

Ultraviolet radiation measurements

Incident solar UV-B radiation was measured from June 9 to June 14, 1993, approximately every 15 min in the 290- to 320-nm range ($W \cdot m^{-2}$) at 0.5-nm intervals, using a Brewers spectrophotometer (SCI-TEC Instruments Inc.) placed at Saskatoon, Sask.

Chemical analyses

Alkalinity and pH samples were analyzed by the National Hydrology Research Institute's Central Chemistry Laboratory, Saskatoon, Sask., using a Metrohm automatic titrator with H_2SO_4 to the appropriate end points. These data were used in the calculations of total CO_2 for primary productivity. Since Redberry Lake microbial growth has been reported to be N and P limited (Waiser and Robarts 1995), the experimental containers were nutrient enriched.

Bacterial enumeration

Bacterial density was measured at the beginning and at the end of each experiment, following the method of Porter and Feig (1980). Samples were stained with DAPI, filtered through 0.2-µm pore-size black Nuclepore filters, and counted under epifluorescence at 1250× using a Zeiss microscope.

Electron transport system activity measurements

Electron transport system (ETS) activity was measured as the rate of reduction of the artificial electron acceptor, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT), to its reduced form, INT-formazan (Packard 1971). Analyses of ETS activity followed the methods of Del Giorgio (1992) and Rai (1984). Plankton samples from each exposure container were incubated daily in Whirl-Pak bags and concentrated on Nuclepore 0.4-µm pore-size polycarbonate membranes (25 mm diameter) under very low vacuum pressure after the incubation period. The homogenation buffer, termination solution, substrate buffer, and INT solution were those used by Rai (1984); the latter two were prepared daily to avoid bacterial contamination. The volume of water filtered was 200 mL, and three separate filters were prepared for each sample of water. Each filter was placed in a plastic tube with 2 mL of homogenation buffer, kept in an ice bath, and sonicated for 3 min, at around 300 W. A 1-mL aliquot was taken from each cell homogenate and placed in a tube with 3 mL of substrate buffer and 1 mL of INT solution. The samples were then incubated for 10 min at in situ temperature. Reaction was stopped by adding 1 mL of termination solution. The samples were then centrifuged for 10 min at 5000 rpm, and the absorbance of an aliquot was read in a spectrophotometer at 490 nm. Blanks for the absorbance readings were made by adding 1 mL of homogenation buffer to 3 mL of substrate buffer and 1 mL INT solution, incubating the mixture for 10 min, and stopping the reaction with 1 mL of termination solution. The absorbances of the samples were converted to rates of oxygen reduction ($\mu g\ O_2 \cdot L^{-1} \cdot h^{-1}$) using the equation given by Rai (1984).

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Chlorophyll *a* analysis

Chlorophyll *a* measurements were carried out by passing 300-mL water samples through a two-stage filter cascade to separate phytoplankton into two size fractions. Nuclepore 45 mm diameter filters (2-μm pore size) and 45-mm Whatman GF/F glass fiber filters (approximately 0.7-μm pore size) were used, resulting in particle fractions of 0.7–2 and >2 μm. The sum of the two fractions was considered, however, for the 16-m sample at the beginning of the first experiment (June 9). Samples were vacuum filtered at around 450 mmHg (1 mmHg = 133.322 Pa). Determinations were done, following Nusch (1980), by boiling 90% ethanol and correcting for pheopigments. Readings were made with a Turner Designs fluorometer and the calculations were carried out according to Strickland and Parsons (1972).

Primary productivity measurements

Primary productivity was measured at the beginning and at the end of the two experiments with the exception of the 16-m containers from the first experiment, which were not considered here. Water samples from the –UV-B and +UV-B containers were incubated in situ for 4 h in BOD bottles and Whirl-Pak bags. Incubations were carried out on four BOD bottles and four Whirlpak bags, which were covered with neutral screens to obtain 4, 45, 60, and 100% of the natural incident PAR. A buoy system was deployed in situ to place the bottles and bags horizontally at approximately 5 cm below the water surface. Polyethylene bags have been used to test the effects of UVR on planktonic organisms (Karentz and Lutze 1990), because they allow the passage of a high percentage of the incident UVR. Approximately 20% of PAR, 25% of UV-A, and 35% of UV-B were filtered by the Whirlpak bags, whereas around 10, 20, and 85% of PAR, UV-A, and UV-B, respectively, were filtered by the BOD bottles (Fig. 1). There exists some controversy in the literature concerning the toxic effect of Whirl-Pak polyethylene bags on phytoplankton (Holm-Hansen and Helbling 1993). This point was extensively discussed by Prézelin et al. (1994), who suggested that this material is not toxic and should be adequate for phytoplankton incubations. We followed the criteria of the latter authors.

The sample volume for photosynthesis rate determinations was 100 mL (bottles and bags), to which 1.48 MBq $NaH^{14}CO_3$ was added immediately prior to the start of incubation. Afterwards, samples were filtered through a two-stage cascade filtering system to separate the particle size fractions of 0.7–2 μm and >2 μm as described for chlorophyll *a* measurements. The filters were then fumed with concentrated hydrochloric acid, dried overnight, and placed in vials with 7 mL of Aquasol scintillation cocktail (NEN Research Products, Boston, Mass.). Samples were counted during 30 min in a scintillation counter. Calculations were made according to the American Public Health Association (1980).

The spectral photoinhibition owing to UV-B (β_λ) was computed to compare the spectral characteristics and the potential effects of the different materials used in this study and natural incident sunlight, following the criteria of Milot-Roy and Vincent (1994):

$$(1) \qquad \beta_\lambda = T_\lambda E_\lambda \varepsilon_\lambda$$

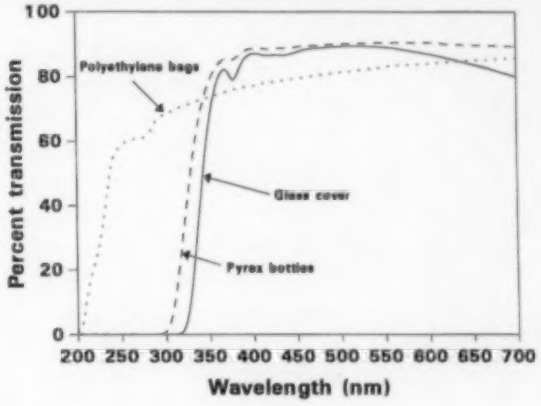
where T is the light transmission through a given material for a given wavelength (λ), E is the incident UV-B flux at that wavelength, and ε is the biological weigthing factor (calculated from the photoinhibition response curve given by Cullen et al. (1992), following the approximations given by Milot-Roy and Vincent (1994)). The relative spectral photoinhibition for the range 290–320 nm (ρ) is

$$(2) \qquad \rho_\lambda = T_\lambda E_\lambda \varepsilon_\lambda / \sum_{290}^{320} (T_\lambda E_\lambda \varepsilon_\lambda) \Delta\lambda$$

Statistical analyses

To test the significance of the responses of phytoplankton to the different UV-B exposure in the experiments, a factorial model of analysis of variance (ANOVA) was used. UV-B exposure (–UV-B and +UV-B containers) and particle size (>2 μm and 0.7–2 μm) were considered as the controlled factors in the first experiment ANOVA. Incubation (BOD bottles and Whirl-Pak bags) was added as a third factor in the analyses of the second experiment. The specific carbon assimilation (the carbon assimilation normalized to chlorophyll *a*, P_B; μg C·μg Chl a^{-1}·h^{-1}) measured at the four PAR intensities was considered as the dependent variable in the analyses, and the PAR percentage was introduced in the model as the covariate. Data were square-root transformed, as the shape of the carbon assimilation versus PAR percentage curves closely followed a quadratic trend. This also allowed the data to meet the normality and homoscedasticity requirements of the ANOVA. Untransformed data are, however, presented in the tables. The differences between initial (t_0) and final (t_f) measured values were used as data entries for mean comparisons. Data corresponding to the two studied depths were considered separately, and statistical analyses were carried out using the SYSTAT for Windows program (SYSTAT Inc. 1992).

Fig. 1. Percentage of light transmission between 200 and 700 nm for the different materials used in the incubations: glass cover, BOD Pyrex bottles, and polyethylene Whirl-Pak bags.



Results

Ultraviolet-B light environment

Incident UV-B radiation revealed clear changes throughout the experiments with maximum values measured at 300 nm on June 10 and 14 (around 4×10^{-4} mW·cm^{-2}), and minimum values on June 12 (near 0.5×10^{-4} mW·cm^{-2}) (Fig. 2). A comparison of the spectral characteristics of the filters used during our experiments is presented. The spectral distribution of incident solar UV-B radiation corresponding to June 14 at noon is represented in Fig. 3*a*, where irradiance at 320 nm was set at 1. The UV-B transmission values of the different materials used in the incubations are included in the figure. The UV-B transmitted by the Whirl-Pak polyethylene bags showed a trend similar to that of the incident UV-B spectral irradiance. In contrast, UV-B transmission through the glass cover and the BOD bottles was markedly lower. However, the glass cover screened out the UV-B radiation more efficiently than the BOD bottles. The values corresponding to the computed biological weighting factor are also included in Fig. 3*a*. The cumulative photoinhibition response showed marked differences when the incident UV-B radiation and the glass cover filter were compared (Fig. 3*b*), with the higher photoinhibition

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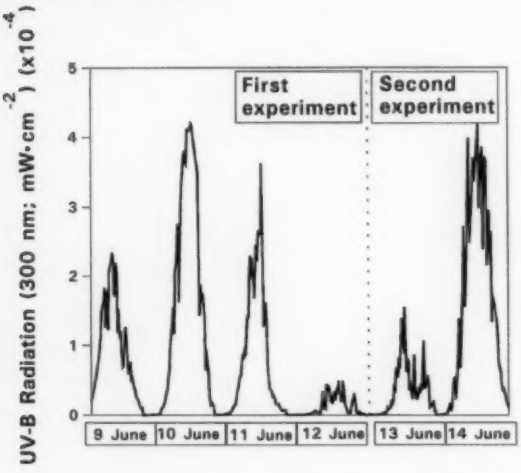
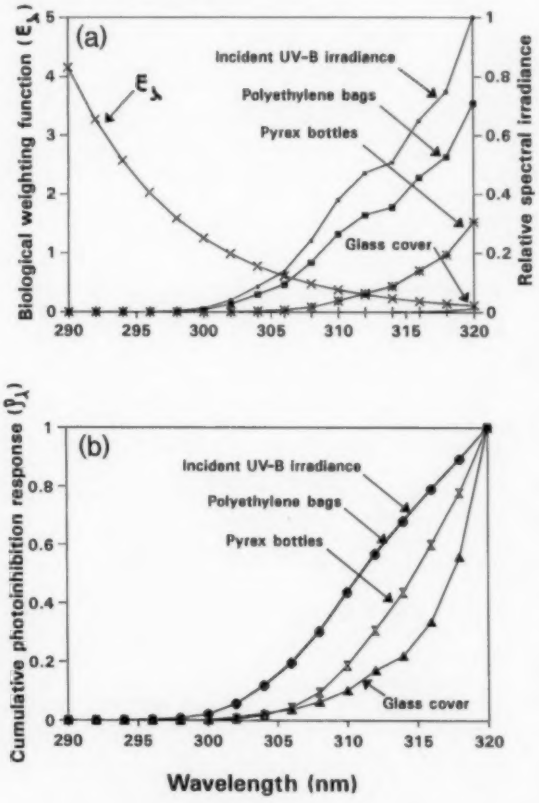
Fig. 2. Daily variation of the incident UV-B radiation at Redberry Lake between June 9 and 14.



values corresponding to the incident UV-B radiation. The same trend can be observed for the comparison between the Whirl-Pak bags and the BOD bottles (Fig. 3*b*). In contrast, the photoinhibition response induced by incident UV-B radiation and by that passing through the Whirl-Pak bags presented identical values (Fig. 3*b*).

Fig. 3. (*a*) Relative spectral irradiance corresponding to the incident UV-B radiation measured at noon on June 14, and the different materials used in the incubations (glass cover, BOD Pyrex bottles, and polyethylene Whirl-Pak bags). The value corresponding to 320 nm of incident UV-B irradiance was set at 1, and the computed biological weighting factor (ε_λ) is included in the figure. (*b*) Cumulative photoinhibition response ($\rho\lambda$) corresponding to the incident UV-B radiation measured at noon on June 14, and the different materials used in the incubations (glass cover, BOD Pyrex bottles, and polyethylene Whirl-Pak bags).



Bacterial abundance

Bacterial density displayed a similar pattern during the two experiments. In both cases cell number increased markedly in the containers exposed to the lower UV-B dose, as compared with the +UV-B and T_o containers (Figs. 4*a* and 4*b*). These differences were in general stronger during the first experiment than during the second one. Moreover, bacterial abundance in the 16-m samples from the first experiment presented maximum differences between –UV-B and +UV-B containers (Fig. 4*a*).

Phytoplankton chlorophyll *a*

First experiment

The chlorophyll *a* concentration increased slightly between June 10 and 12 at the two depths studied (Figs. 5*a* and 5*b*). However, the chlorophyll *a* concentration from the 16-m +UV-B container increased markedly when compared with the rest of the treatments (Fig. 5*b*). Furthermore, the T_o values corresponding to June 9 from 16 m presented a higher pigment concentration than the two experimental containers on June 10 (5.4 times higher than the UV-B and 2.4 times higher than the +UV-B tanks, respectively) (Fig. 5*b*). Chlorophyll *a* size fractionation showed that in general the smallest fraction (0.7–2 µm) was quantitatively the most important despite the observation that the >2-µm fraction became significant on June 12 in the 16-m samples. Pheopigment concentration (data not shown) was always low.

Second experiment

No major changes in chlorophyll *a* concentration were noticed for the 4-m containers. The smallest fraction was the dominant one in both samples (–UV-B and +UV-B) (Fig. 5*c*). In the 16-m samples chlorophyll *a* concentration showed a marked decrease between the beginning and the end of the experiment (Fig. 5*d*). Similar observations were made during the first experiment, between June 9 and 10 (Fig. 5*b*). Changes in the particle size were also observed, with the chlorophyll *a* size fraction >2 µm higher in the T_o container than in the –UV-B and +UV-B containers.

Specific carbon assimilation

The ANOVA did not show a significant effect for the covariate (percentage of incident PAR) in either of the two experiments carried out.

First experiment

No UV-B exposure effect on 4-m phytoplankton photosynthesis could be observed, as shown by the ANOVA ($P > 0.05$; Table 1). The differences between T_f and T_o carbon assimilation,

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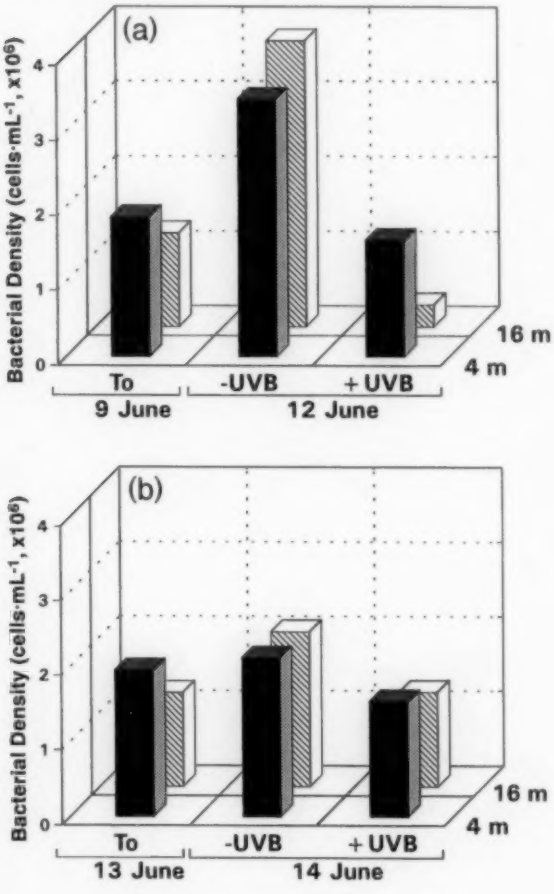
Fig. 4. Number of bacteria corresponding to experiments 1 (*a*) and 2 (*b*). T_o denotes the start of the experiments, and –UV-B and +UV-B represent the experimental containers.



however, were negative and significantly higher for the smallest particles (0.7–2 µm) than for the larger fraction (>2 µm) ($P < 0.001$; Table 1). On the other hand, no interaction between UV-B exposure and particle size was found ($P > 0.05$; Table 1).

Second experiment

Significant UV-B exposure effects were found for 4-m phytoplankton ($P < 0.01$). The difference in specific carbon assimilation was lower in the +UV-B containers than in the –UV-B ones (Table 2). This result contrasted with the 16-m samples, for which no statistical difference between treatments was observed ($P > 0.05$; Table 3). For the incubation treatments (bottle and bag), no effect was observed for either of the two depths. In addition, no interaction between UV-B exposure and incubation ($P > 0.05$; Tables 2 and 3) was found. The particle size factor displayed the same behaviour as in the first experiment for both depths: carbon assimilation was significantly reduced in the case of the smallest particles compared with the larger ones ($P < 0.01$ and $P < 0.05$ for 4 and 16 m, respectively; Tables 2 and 3). In addition, no significant effect

Table 1. Results of the factorial ANOVA for phytoplankton from 4 m during the first experiment (June 10–12).

	Difference between t_f and t_o measurements of P_B	F	P
Main effects			
UV-B exposure			
–UV-B container	–0.85±0.41	1.16	0.305
+UV-B container	–1.22±0.49		
Particle size			
0.7–2 µm	–2.09±0.27	48.44	<0.0001*
>2 µm	–0.02±0.17		<0.001*
First-order interactions			
UV-B exposure vs. particle size			
–UV-B, 0.7–2 µm	–1.81±0.35	0.09	0.769
–UV-B, >2 µm	–0.11±0.18		
+UV-B, 0.7–2 µm	–2.37±0.41		
+UV-B, >2 µm	–0.08±0.31		

Note: Values are the mean ± SE of the differences between final and initial ($t_f - t_o$) measurements of P_B (µg C·µg Chla^{-1}·h^{-1}) for the treatments. Main effects and first-order interactions are represented. The controlled factors are UV-B exposure (–UV-B and +UV-B containers) and particle size (phytoplankton of 0.7–2 µm and >2 µm).

*Significant difference between treatments.

was found between UV-B exposure and particle size interactions ($P > 0.05$; Tables 2 and 3). However, a significant interaction was noticed for incubation versus particle size treatments in 4-m phytoplankton ($P < 0.05$). This can be explained by the different responses of the two size fractions in bottle and bag incubations. Carbon assimilation by >2-µm cells was lower in bag than in bottle incubations, whereas the opposite trend was displayed by 0.7- to 2-µm phytoplankton (Table 2). No interaction was found between the latter factors for 16-m samples ($P > 0.05$; Table 3).

Electron transport system and respiration

Daily measurements of the ETS activity displayed a similar pattern during the two experiments, presenting the highest ETS values in the +UV-B containers (Figs. 6*a*–6*d*). The ETS activity showed an increasing trend as a function of time, except for the –UV-B containers on June 9 and 10 (Figs. 6*a* and 6*b*). ETS activity in the +UV-B containers was higher than that observed in the –UV-B ones for all of the experimental conditions. These differences were more pronounced for the second experiment and in the community corresponding to 16 m in experiment 1. They were slight for the 4-m subsample during the latter experiment. An ANOVA of two factors (days and UV-B exposure) for the 4-m first experiment data, however, showed significant differences between days ($P < 0.05$) and between –UV-B and +UV-B containers as well ($P < 0.05$ for 4 m and $P < 0.001$ for 16 m).

Discussion

Phytoplankton chlorophyll *a* concentration and bacterial abundance

The changes in chlorophyll *a* concentration showed the existence of a period of adaptation to the experimental conditions, which was more marked for the phytoplankton collected from

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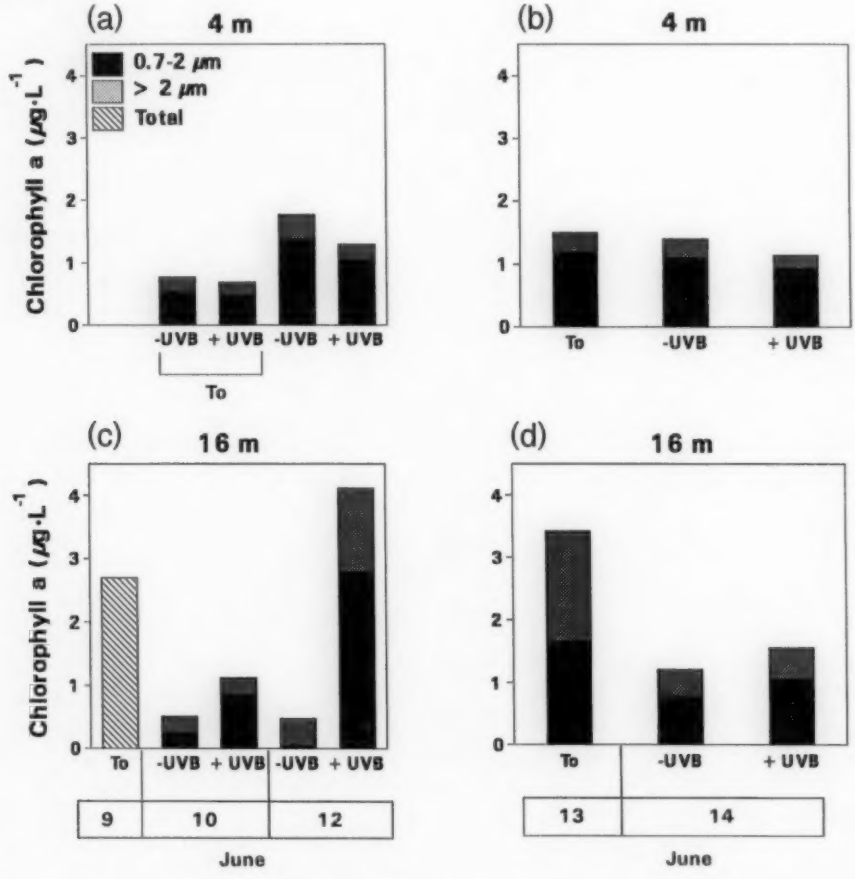
Fig. 5. Chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) from the two experiments: 4 m, experiment 1 (*a*); 16-m, experiment 1 (*b*); 4-m, experiment 2 (*c*); 16-m, experiment 2 (*d*). T_o denotes the start of the experiments, and –UV-B and +UV-B represent the experimental containers. Note that in all cases the phytoplankton size fractions of 0.7–2 µm and >2 µm have been represented with the exception of T_o on June 9, corresponding to the total amount of chlorophyll *a*.



the 16 m depth (Figs. 5*b* and 5*d*). The chlorophyll *a* concentration increased continuously in the 16-m +UV-B containers, with the highest values occurring towards the end of the first experiment. A possible explanation of these results is a decrease in the grazing pressure by small grazers, which allowed a high growth rate of phytoplankton. These organisms could not be completely separated at the beginning of the experiment by the Nitex mesh used and were probably less resistant to UV-B in the 16-m samples. This observation is similar to that of Bothwell et al. (1994), who suggested that effects of UV-B are significant not only for algae but also, and perhaps more importantly, for higher trophic levels. As we have no samples for zooplankton counts, no data are available to test this hypothesis.

A clearer pattern was displayed by the changes in bacterial abundance, with a marked reduction of cell number under +UV-B conditions during both experiments (Figs. 4*a* and 4*b*), thus suggesting the presence of a high sensitivity of Redberry Lake bacteria to UV-B exposure. These results are coincident with those of Ferguson and Palumbo (1979) for neritic waters and those of Thomson et al. (1980) for estuarine bacteria.

Specific carbon assimilation

A complex pattern was displayed by the response of phytoplankton to UV-B exposure. During the second experiment, a significant reduction in P_B related to UV-B exposure was observed for 4-m phytoplankton. The fact that during the first experiment the responses in the –UV-B and the +UV-B containers for the same depth were similar could signify that phytoplankton reacts to UV conditions within the time scale of a day, since the UV-B environment was markedly different at the end of the two experiments: higher irradiation was received by the cells on June 14 than on June 12 (Tables 1 and 2).

Phytoplankton photosynthesis has been reported to be partially inhibited by UV-B when incubated in Whirl-Pak bags, as compared with Pyrex bottle incubations. This results from the low screening of UV-B by polyethylene (Smith et al. 1992). Nevertheless, our results did not show significant differences in specific carbon assimilation between bottle and bag incubations during the second experiment for either depth (Tables 2 and 3). This could signify that UV-B had little effect on Redberry phytoplankton short-term incubations (hours).

Smaller cells (0.7–2 µm) were in general more affected

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Table 2. Results of the factorial ANOVA for phytoplankton from 4 m during the second experiment (June 13–14).

	Difference between t_f and t_o measurements of P_B	F	P
Main effects			
UV-B exposure			
–UV-B container	–1.39±0.53	11.09	0.003*
+UV-B container	–2.86±0.46		
Incubation			
Bottle	–2.08±0.63	0.01	0.977
Bag	–2.17±0.42		
Particle size			
0.7–2 µm	–3.06±0.47	8.06	0.009*
> 2 µm	–1.19±0.48		
First-order interactions			
UV-B exposure vs. incubation			
–UV-B, bottle	–1.01±1.02	2.30	0.143
–UV-B, bag	–1.78±0.37		
+UV-B, bottle	–3.15±0.55		
+UV-B, bag	–2.56±0.76		
UV-B exposure vs. particle size			
–UV-B, 0.7–2 µm	–2.63±0.51	1.82	0.191
–UV-B, >2 µm	–0.16±0.73		
+UV-B, 0.7–2 µm	–3.49±0.80		
+UV-B, >2 µm	–2.23±0.39		
Incubation vs. particle size			
Bottle, 0.7–2 µm	–3.66±0.50	9.08	0.006*
Bottle, >2 µm	–0.50±0.85		
Bag, 0.7–2 µm	–2.46±0.77		
Bag. >2 µm	–1.88±0.38		

Note: Values correspond to the mean ± SE of the differences between final and initial ($t_f - t_o$) measurements of P_B (µg C·µg Chla^{-1}·h^{-1}) for the treatments. Main effects and first-order interactions are represented. The controlled factors are UV-B exposure (–UV-B and +UV-B containers), incubation (BOD bottle and Whirl-Pak incubations), and particle size (phytoplankton of 0.7–2 µm and >2 µm).

*Significant difference between treatments.

Table 3. Results of the factorial ANOVA for phytoplankton from 16 m during the second experiment (June 13–14).

	Difference between t_f and t_o measurements of P_B	F	P
Main effects			
UV-B exposure			
–UV-B container	0.40±0.42	0.90	0.354
+UV-B container	–0.22±0.31		
Incubation			
Bottle	–0.03±0.34	0.15	0.698
Bag	0.21±0.41		
Particle size			
0.7–2 µm	–0.40±0.43	5.85	0.024*
>2 µm	0.58±0.27		
First-order interactions			
UV-B exposure vs. incubation			
–UV-B, bottle	0.09±0.53	0.86	0.364
–UV-B, bag	0.71±0.68		
+UV-B, bottle	–0.16±0.46		
+UV-B, bag	–0.28±0.44		
UV-B exposure vs. particle size			
–UV-B, 0.7–2 µm	0.03±0.72	0.06	0.804
–UV-B, >2 µm	0.77±0.46		
+UV-B, 0.7–2 µm	–0.82±0.47		
+UV-B, >2 µm	0.38±0.30		
Incubation vs. particle size			
Bottle, 0.7–2 µm	–0.55±0.54	0.25	0.624
Bottle, >2 µm	0.49±0.35		
Bag, 0.7–2 µm	–0.24±0.70		
Bag, >2 µm	0.67±0.43		

Note: Values are the mean ± SE of the differences between final and initial ($t_f - t_o$) measurements of P_B (µg C·µg Chla^{-1}·h^{-1}) for the treatments. Main effects and first-order interactions are represented. The controlled factors are UV-B exposure (–UV-B and +UV-B containers), incubation (BOD bottle and Whirl-Pak incubations), and particle size (phytoplankton of 0.7–2 µm and >2 µm).

*Significant difference between treatments.

than the larger ones (>2 µm), as shown by the main effects of the ANOVA for the two experiments (Tables 1–3). The lack of interaction between UV-B exposure and particle size and the lower values for 0.7- to 2-µm phytoplankton within the two UV-B treatments suggest that the different responses of the two size-classes could be more related to the experimental conditions imposed in the containers than to UV-B treatments. However, the significant interaction between incubation and particle size factors during the second experiment for 4-m phytoplankton illustrates a different response by the two size fractions to bottle and bag incubations. Larger cells were observed to be less adapted to bag incubations than the smaller ones. These results indicate that phytoplankton composition in the 4-m samples would be a determining factor in our study.

In contrast to the 4-m samples, the response of 16-m phytoplankton to UV-B was found to be more related to the time of adaptation to the experimental conditions than to the specific composition of algae (Table 3). The lack of difference between the –UV-B and +UV-B containers during the second experiment coincided with a drastic decrease in chlorophyll *a* concentration from June 13 to 14. These changes were similar to those observed between June 9 and 10, at the beginning of the first experiment, suggesting that 16-m algae required at least 24 h to adapt to the imposed conditions. In addition, 16-m phytoplankton presented marked variations in the chlorophyll *a* concentration, probably related to changes in the grazing pressure within the +UV-B container, as mentioned above.

Despite the variable responses of surface phytoplankton P_B during the two experiments, short-term incubation in bottles and bags did not affect carbon assimilation in 4- and 16-m phytoplankton adapted to –UV-B and +UV-B conditions. These results can be explained by the existence of internal and (or) external photoprotective mechanisms. The former involve the synthesis of UV-B-absorbing substances and carotenoids. Mycosporine-like amino acids (water-soluble imino-carbonyl derivatives of mycosporines), the most studied sunscreen substances, have their maximum absorption in the range of 310–360 nm (UV-A and UV-B). The photoprotective function of these substances has been suggested for a variety of environments and organisms (Shibata 1969; Dunlap et al. 1986; Karentz et al. 1991; Vincent and Roy 1993). Carotenoids act as accessory pigments via absorption and transfer of excitation

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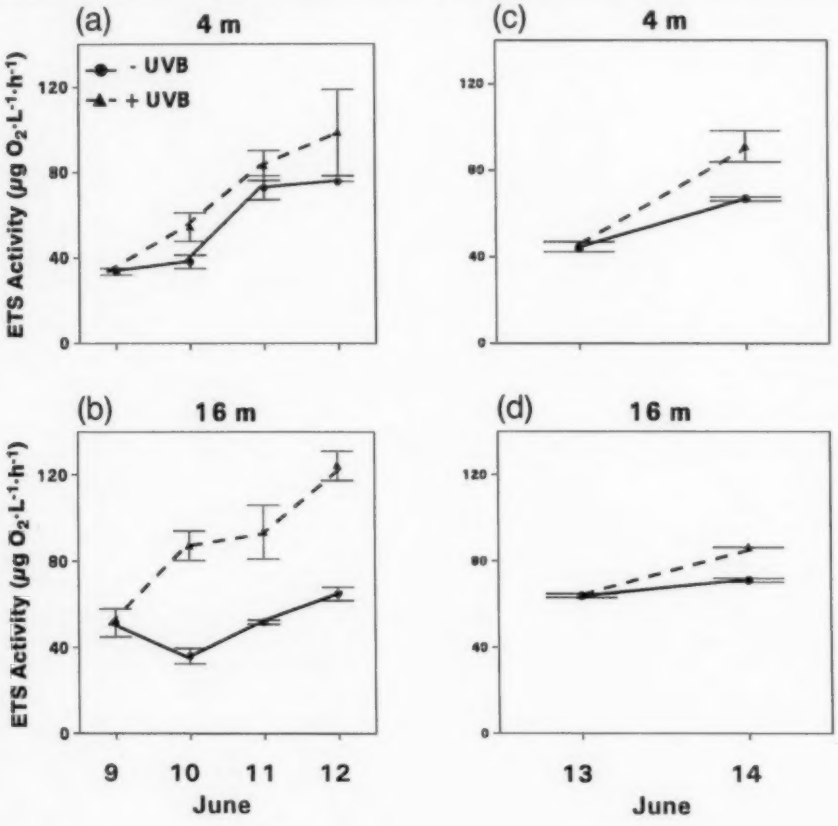
Fig. 6. Changes in ETS activity (μg $O_2 \cdot L^{-1} \cdot h^{-1}$) during the first experiment (*a*, *b*) and the second experiment (*c*, *d*) for phytoplankton from 4 and 16 m depth. Circles denote incubations in –UV-B containers and triangles indicate incubations in +UV-B containers. Error bars are ±1SE.



energy towards chlorophyll *a* in the 400- to 550-nm range of PAR. They also protect against photooxidation of chlorophyll *a* and other molecules (Clayton 1966, 1980; Paerl et al. 1983). Phytoplankton of Redberry Lake could have developed some of these adaptive responses. However, we lack the information to support this hypothesis. On the other hand, a possible interpretation of the observed results could be the high DOC concentrations in Redberry Lake (mean for 2 m depth is 35.3 $mg \cdot L^{-1}$ and for 16 m it is 37.4 $mg \cdot L^{-1}$; Tumber et al. 1993). The 300-nm absorption coefficient calculated at the time of our experiments (H. Maske, CICESE, Apdo. Postal 2732, Ensenada, Baja California, Mexico. e-mail: hmaske@cicese.mx, personal communication) was as high as 10 m^{-1}, meaning that the 1% UV-B depth was located at around 40 cm and 55% of UV-B was absorbed by DOC at the depth of our primary production incubations (5 cm). Consequently, they could act as an environmental screen that could bring photoprotection against UV-B damage on phytoplankton cells.

Electron transport system activity

The elevated values of ETS activity measured in the +UV-B containers during our experiments, as compared with the -UV-B containers, suggest a significant increase in respiration at the community level (Figs. 6*a*–6*d*). Similar experimental results were found by Thomson et al. (1980) for estuarine bacteria. Recently published information showed disparate responses in growth and biomass of bacteria in relation to UV-B-induced modifications in the DOC pool (Herndl et al. 1993; Lindell et al. 1995). Most of the DOC pool present in lakes is composed of refractory humic substances that are partially available to bacteria (Tulonen 1993). These compounds can be degraded by photochemical interaction with UV-B (Zepp 1982; Kieber et al. 1990; Vincent and Roy 1993), thus resulting in an increase in the concentration of low molecular weight DOC available for heterotrophy. Our results of ETS activity in the +UV-B containers seem to support this hypothesis. However, the time scale of photolysis of DOC by UV-B in Redberry Lake is longer than the duration of the experiments (>1 day, <6 days; R.D. Robarts and M.J. Waiser, NHRI, 11 Innovation Blvd., Saskatoon, SK S7N 3H5, personal communication), which significantly weakens the above hypothesis.

An alternative explanation of the observed increase in ETS activity under +UV-B conditions would be related to an augmentation in the energetic cost involved in the generation of internal mechanisms of photoprotection. Smaller cells, which were the dominant fraction in Redberry Lake (mostly picocyanobacteria; W.F. Vincent, Département de biologie, Université Laval, Sainte-Foy, QC G1K 7P4, personal communication), have to invest a high proportion of their energy budget in the synthesis of photoprotective substances to reach significant

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levels of tolerance to UV-B (Garcia-Pichel 1994). Another important mechanism of photoprotection is the synthesis of enzymatic antioxidants. These compounds may be relevant in environments with high DOC concentrations, like Redberry Lake. Interactions between UV-B and DOC can lead to the formation of hydrogen peroxide (H_2O_2) in the water column. H_2O_2 diffuses through the cell membrane and can also be formed inside the cells, liberating reactive oxygen species. These species are highly damaging for the cells. Aquatic organisms have developed protective strategies, like the synthesis of superoxide dismutase (SOD) and catalase, which can act as detoxification mechanisms. The activity of these enzymes has been recently described for marine invertebrates (Abele-Oeschger et al. 1994) and freshwater algae (Malanga and Puntarulo 1995). However, we do not have the information to validate these hypotheses, and more research is required to assess our knowledge of these important points.

The data set presented here is rather limited, particularly owing to the lack of information on underwater light spectra. However, our results suggest that DOC may play a significant role in the dynamics of the planktonic community of Redberry Lake. Future work on the effects of UVR in prairie lake ecosystems should involve the integrated analysis of the different food web components, as well as the study of biological weighting functions and the spectral distribution of UVR in the water column. Furthermore, special attention should be paid not only to effects at the population and the community levels, but also to the UV-B interaction with the nonliving components of the ecosystem. These nonliving components could be substantially modified and thus interact with the biota by indirect pathways. This approach would give a more realistic understanding of the processes involved.

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An evaluation of techniques for measuring periphyton metabolism in chambers

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Abstract: Experiments were performed to compare ^{14}C uptake, dissolved O_2 and CO_2 change, and microelectrode techniques for measuring benthic primary productivity and community metabolism at a site on the South Saskatchewan River, Saskatchewan. The highest estimates of primary productivity were obtained with O_2 microelectrodes (two to four times higher than simultaneous estimates from bulk-water dissolved O_2 measurements), presumably because measurements are biased toward active communities under optimal conditions for photosynthesis. In daytime experiments lasting ~1.3 and 2.4 h, estimates from ^{14}C uptake were higher than those measured simultaneously from dissolved O_2 and CO_2 change, suggesting that ^{14}C uptake measured gross primary production (GPP) in these short-term incubations. Differences in experimental water velocity ranging from 0.2 to >6.5 $cm \cdot s^{-1}$ had less of an effect than chlorophyll *a* concentration on metabolic rates in diel experiments with rocks taken from a site where velocities were from 5.6 to 7.8 $cm \cdot s^{-1}$. There was a potential for phosphorus and possibly NH_4 limitation of metabolic activity during long-term incubations. Results highlight areas in which further research is needed when using chambers to determine periphyton metabolism.

Résumé : Nos expériences visaient à comparer l'assimilation du ^{14}C, les changements dans l'O_2 dissous et le CO_2 et les techniques faisant appel à des microélectrodes pour mesurer la productivité primaire benthique et le métabolisme des communautés sur un site de la rivière Saskatchewan sud (Saskatchewan). Ce sont les microélectrodes à O_2 qui ont donné les estimations les plus élevées de la productivité primaire (deux à quatre fois plus élevées que les estimations simultanées des mesures de l'O_2 dissous dans l'eau libre), ce qui est probablement dû au fait que les mesures sont biaisées en faveur des communautés actives dans les conditions idéales de la photosynthèse. Dans des expériences en lumière du jour d'une durée de ~1,3 et 2,4 h, les estimations établies à partir de l'assimilation du ^{14}C étaient plus élevées que celles fournies par les mesures simultanées du changement de l'O_2 dissous et du CO_2, ce qui permet de penser que l'assimilation du ^{14}C mesurait la production primaire brute dans ces incubations de courte durée. Les différences dans la vitesse de l'eau pendant les expériences, de l'ordre de 0,2 à >6,5 $cm \cdot s^{-1}$, avaient moins d'effet que la concentration de chlorophylle *a* sur le métabolisme dans les expériences diurnes réalisées avec des roches prélevées sur un site où les vitesses étaient de l'ordre de 5,6 à 7,8 $cm \cdot s^{-1}$. Les incubations de longue durée présentaient un potentiel de limitation du métabolisme du phosphore et peut-être du NH_4. Les résultats font ressortir les domaines où il est nécessaire de poursuivre les recherches quand on utilise des enceintes pour mesurer le métabolisme du périphyton.
[Traduit par la Rédaction]

Introduction

Primary productivity is a significant component of stream and river energy budgets, with periphyton often making a greater contribution to biomass and metabolic activity than phytoplankton (Bott 1983). While periphyton production can also be important in the littoral of lakes and in estuaries, probably nowhere are the practical problems associated with measuring periphyton metabolism more difficult than in lotic systems.

Periphyton production in streams and rivers is generally estimated from the change in O_2 or CO_2 content of a stream segment or a particular stream community enclosed in a test chamber. Measurements based on open-system changes in dissolved oxygen (DO; Odum 1956) or CO_2 (Wright and Mills 1967) require accurate determination of reaeration. Diffusion across the air–water interface can be difficult to quantify, especially if a study reach has nonuniform hydraulic characteristics; sometimes the correction applied is much greater than

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the community metabolic rate (Bott et al. 1978). Techniques to measure reaeration using tracer compounds have become available, but this approach involves sophisticated instrumentation (Kilpatrick et al. 1989). Therefore, many investigators have chosen to measure metabolism by transferring communities to metabolism chambers (McIntire and Phinney 1965; Sumner and Fisher 1979; Bott et al. 1985). However, transferring biological material from its native lotic habitat to chambers raises concerns related to changes in water velocity (usually to a lower level) and irradiance from those occurring in situ, nutrient depletion, and problems with O_2 or CO_2 bubble formation under supersaturated conditions. Broader issues involve the replication required to allow estimation of reach metabolism from a limited number of community samples and the degree to which those samples are representative of the range of substrates found in the reach.

In addition to gas change techniques, [^{14}C]bicarbonate uptake also has been used to measure lotic periphyton productivity (Naiman 1976; Hill and Webster 1982; Hornick et al. 1981). However, although the indicated studies are exceptions, few studies have compared this method with gas change procedures (Hunding and Hargrave 1973; Turner et al. 1983), and fewer still have compared measures made simultaneously on the same sample in the field (Bott and Ritter 1981). The finest spatial resolution of periphyton metabolism is achieved by making measurements with O_2 microelectrodes. Microelectrode measurements require highly active communities as well as stable and high light intensities. Revsbech et al. (1981) compared ^{14}C uptake and bulk-water DO change with O_2 microelectrode measurements, but such comparisons are rare.

The aims of this study were (*i*) to compare the bulk-water DO and CO_2 change, ^{14}C uptake, and microelectrode methods for measuring metabolism using a natural periphyton community exposed to ambient river conditions and (*ii*) to address some of the concerns related to the use of chambers for measuring periphyton metabolism, specifically the effect of water velocity and nutrient depletion on measurements of metabolism.

Study site

The study site (52°8′N, 106°38′W) was on the South Saskatchewan River ~19 km north of Saskatoon, where the river is seventh order, approximately 200 m wide, and enriched with nutrients from the Saskatoon sewage treatment plant as well as runoff from feedlots and cropland. During 1993, the river was ice free for 2 months prior to the research. River discharge is regulated by a dam approximately 114 km upstream of Saskatoon and measurements at Saskatoon ranged from 131 to 164 $m^3 \cdot s^{-1}$ during the study period (June 9–12). Reflecting the heterogeneity of the stream bed, samples used in experiments were collected from two habitats: (*i*) a shallow (~0.15 m deep) sand–silt bed near the river edge, and (*ii*) a riffle (0.4–1.0 m deep) with a cobble-dominated riverbed.

Materials and methods

Community metabolism measurements

Chambers

Clear acrylic chambers of three designs (Aliquot, Boise, Idaho) were used: (*i*) cylinders (2.95 L; 15 cm i.d. × 45.5 cm long) equipped for water recirculation with a submersible pump (model No. 893-001-04, March Manufacturing Co., Glenview, Ill.), (*ii*) small rectangular chambers (3.9 L; 12 cm wide × 39 cm long × 5 cm deep, inside dimensions), and (*iii*) large rectangular chambers (11.3 L; 20 cm wide × 56 cm long × 10 cm deep). The rectangular chambers had three aluminum-framed 2.5 cm thick polycarbonate flow diffusers, a silicone O-ring seal on the top and bottom, and PVC bases. Water in the rectangular chambers was recirculated with several types of submersible 120-V AC pumps (maximal discharge rate of 50 $L \cdot min^{-1}$). A globe Y valve was used to control water velocity. A shunt valve directed a portion of the chamber circulation past a DO probe.

Metabolism experiments were conducted in situ in an unshaded location a few metres from the river bank. The chambers were suspended in the water column on adjustable-height tables constructed of aluminum-framed fiberglass-reinforced plastic grating. Hand-operated winches on each corner of the 1.3 × 1.4 m tables enabled the chambers to be positioned in the water column for incubation (~0.25 m water depth) and raised above the water surface for setup. A polyethylene head tank (25-L capacity) connected to each chamber with flexible PVC tubing was used to hydrostatically test the chambers for leaks. The tank was equipped with a diffuser into which N_2 gas could be injected for the purpose of stripping DO to control supersaturation.

Experiment 1: comparison of bulk-water DO and CO_2 change and ^{14}C uptake procedures

Three runs were performed on two bright, sunny days (June 9 and 10) using sediment communities collected from the river margin. Sediment samples were collected with a small coal shovel to a depth of ~4 cm with negligible disturbance of the epilithic periphyton mat, transferred to aluminum trays (100 or 108 cm^2), and placed in the chambers. The experiment on June 9 was performed using one light and one dark cylindrical chamber and on June 10 with single light and dark chambers of both cylindrical and small rectangular design. Water samples were collected for DO, total alkalinity, pH, NH_4, $NO_3 + NO_2$, and soluble reactive phosphorus (SRP) analyses prior to the start of the experiment. After chambers were filled with water and sealed, [^{14}C]bicarbonate (specific activity 2072 $mBq \cdot mmol^{-1}$; Amersham Radiolabeled Chemicals, St. Louis, Mo.) was added to each chamber to provide a final concentration of 3.7 $kBq \cdot mL^{-1}$; chambers for dark incubations were covered immediately with black plastic. Incubations were stopped by gluteraldehyde addition (1% final concentration) before any significant accumulation of gas bubbles on the walls of the light chambers occurred. Experiments lasted ~1.3 h on June 9 and ~2.4 h on June 10. Postincubation water samples were collected from each chamber for measurement of DO, total alkalinity, and excreted radioactivity. After the incubation water was drained, periphyton and sediment (to 3 mm depth) samples were scraped into containers, fixed with 1% gluteraldehyde, and stored on ice until processing as described below.

Experiment 2: velocity experiment

Diel experiments to measure the effect of water velocity on community metabolic parameters were performed with periphyton-coated rocks (~18–22 cm in diameter) with a relatively flat upper surface. In situ water velocities were measured 1 mm above each rock and at ~0.6 of total depth before removal and placement of the rock in one of the four large rectangular chambers. During runs, we avoided underestimating metabolism resulting from bubble formation (supersaturation) by exchanging chamber water with N_2-bubbled river water (lowering the DO to ~5 $mg \cdot L^{-1}$) and maintaining DO in the chambers in the range of 50–100% saturation. DO concentrations were monitored once a minute, with the 10-min mean recorded automatically with an electronic data logger (CR10, Campbell Scientific, Logan, Utah). At the end of each run, the periphyton were fixed with 1% gluteraldehyde, and the rocks were placed in a cooler for return to the laboratory. The volume of each chamber and associated circulation

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lines was determined by draining the water into graduated cylinders; correction for rock volume was done by water displacement.

Experiment 3: comparison of O_2 microelectrodes and bulk-water DO changes

Oxygen microelectrodes were used to assess rates of photosynthesis and O_2 diffusion for periphyton mats in situ and for cores (108 cm^2 × 2.5 cm deep) placed in the small rectangular chambers. The probe, consisting of a cathode-type microelectrode with a tip diameter of 30 µm embedded in a stainless hypodermic needle, was calibrated to DO concentrations determined by Winkler titrations and to anoxic conditions found in reducing sediments. Primary production was estimated with the light–dark method described in detail by Revsbech et al. (1981) and Revsbech and Jorgensen (1983, 1986). The decrease in DO from a steady-state concentration was determined 1 s after darkening at 1-mm intervals into the mat until rates were undetectable (usually within 3 mm). Volumetric rates were then integrated over the depth of the mat to yield areal estimates. Oxygen diffusion was calculated using DO profiles from the surface of the mat under natural sunlight. The rate of diffusion was then calculated from the steepness of the profile using Fick's Law of diffusion and the DO diffusion coefficient. Microelectrode determinations of DO were made before and after a 2-h incubation and compared with bulk-water DO changes determined from triplicate Winkler determinations of DO before and after the period, as well as with data obtained in experiment 1, which was run concurrently. Water velocity in the chamber was set to equal that 1 mm above the sediment at the site of sample collection. Bulk-water DO change was estimated as described below. The experiment was run twice.

Sample processing

Water samples collected for measurement of ^{14}C-labeled algal excretion products were filtered through 0.45-µm pore size membrane filters, acidified, and bubbled with air to drive off unincorporated inorganic ^{14}C. Subsamples were transferred to Filter Count cocktail (Packard Instruments, Naperville, Ill.) and radioactivity was determined using a liquid scintillation counter (model 1900CA, Packard Instruments).

The periphyton–sediment samples were stirred, and subsamples of known volume were taken for determination of chlorophyll *a* concentration (five replicates), bacterial densities (three replicates), ash free dry mass (AFDM; three replicates), and incorporated radioactivity (five to seven replicates). Samples were dried at 100°C for AFDM and radioactivity determinations. AFDM samples were then combusted at 550°C for 1 h. Subsamples for radioactivity determinations were exposed to HCl fumes under a hood and then combusted in a sample oxidizer in which $^{14}CO_2$ is collected in ethanolamine (model 307, Packard Instruments) prior to liquid scintillation counting.

Periphyton on rocks were scraped, brushed, and washed from the rock surface, and the samples were made up to known volume. Aliquots (10 mL) were filtered onto Whatman GFC filters for chlorophyll *a* concentration (five replicates) and AFDM (three replicates) determinations. Rock planar area was determined by tracing the outline of the top of the rock, cutting out the shape, and comparing its weight with the weight of a known area of paper.

Physical and chemical measurements

Solar radiation was measured with a radiometric sensor and data logger (LI-185 and LI-1000, respectively; LI-COR Instruments, Lincoln, Neb.) 20 m from the river in an unshaded location. Short-wave radiation (SWR) was integrated at 10-min intervals and converted to photosynthetically active radiation (PAR) using an empirical relationship developed for a site in Jenner, Alberta (55°50′N, 111°12′W; PAR (µmol quanta·m^{-2}·s^{-1}) = 2.01 × SWR (W·m^{-2}); J.T. Brock, unpublished data). PAR incident on the chambers was estimated from incubation depth and extinction measurements conducted during the study using an underwater quantum sensor (LI 192; LI-COR Instruments). DO was measured using Winkler titrations (June 9 and 10) or DO probes and meters (YSI model 57 and Orion model 840) calibrated to ambient temperature and pressure and inserted in chamber circulation hoses (June 11–13). Water temperature was determined with a mercury thermometer or thermistors on DO probes; pH was measured with a Fisher Accumet digital pH meter.

Total alkalinity was determined from Gran titrations, and the concentration of free CO_2 was calculated according to Stumm and Morgan (1981). Water samples were collected in Nalgene polyethylene bottles for SRP analyses or in polystyrene containers for NH_4 and $NO_2 + NO_3$ analyses. Samples were held at 4°C until analyzed within 24 h of collection. SRP was analyzed using the molybdenum blue method (Murphy and Riley 1962). $NO_2 + NO_3$ samples were filtered through prewashed 0.45-µm pore size HAWP Millipore membrane filters and analyzed on a Technicon autoanalyzer (Stainton et al. 1977). NH_4 was analyzed spectrophotometrically following the phenol hypochlorite method of Solorzano (1969).

Water velocities were measured in the river where benthic samples were collected and in the chambers. A thermistor flow meter (LaBarbera and Vogel 1976) was used to measure current speeds ~1 and 5 mm above the sediment surface (experiment 1) and 1 mm above the front, middle, and back of each rock (experiment 2). In situ water velocities 5 cm above the river bed and at approximately 0.6 of total depth were determined using a Gurley-Price meter (model 22). The thermistor flow meter malfunctioned during run 2 of experiment 2 and thus the chamber water velocities were estimated from pump discharge and the cross-sectional area of the chamber (accounting for occlusion from the rock face).

Community analyses

Algal biomass and nutrient content

Samples for chlorophyll *a* analyses were frozen for 24–72 h and then extracted in 90% ethanol at 80°C for 5 min. After cooling in the dark for 30 min, chlorophyll *a* concentration was determined fluorometrically (Turner Designs, Sunnyvale, Calif.) with correction for phaeophytin according to Nusch (1980) and Sartory and Grobbelaar (1984).

Intact sediment cores were examined with a Bio-Rad MRC 600 scanning confocal laser microscope (SCLM) attached to a Nikon Microphot SA with a Nikon water immersion objective 40×, 0.55 numerical aperture (NA). Samples for microscopy studies were collected by pressing a plastic syringe with the tip cut off into the sediment, gently withdrawing the syringe with the core inside, and replacing the plunger underneath the core. Using the plunger, the core was moved toward the top of the syringe until 1 mL of water remained and gluteraldehyde was added to fix the sample. In the laboratory, the water was gently removed, and the core was sliced on the diagonal from the center of the core surface to the syringe wall at a 5 mm depth with a scalpel and the slice was removed. During image collection the SCLM was equipped with an *XYZ* position stage controlled by a Northgate 486 computer. Algae were visualized by autofluorescence (Caldwell et al. 1992).

Bacterial densities

Samples for bacterial density determinations were fixed with 0.2 mL 1% gluteraldehyde and refrigerated. Two millilitres of 0.1 M sodium pyrophosphate fixed with formaldehyde was added, and samples were sonicated with a microtip probe (Heat Systems Ultrasonics, Farmingdale, N.Y.) at 45 W for 45 s. After adding 8 mL of 25% glycerol, samples were brought to 2000 × *g* in a centrifuge at 4°C. The supernatant fluid was removed, and the sediment pellet was washed twice with 25% glycerol. An aliquot was removed from the pooled supernatant fluids and stained for 15 min with propidium iodide (20 µg·mL^{-1} final concentration; peak excitation at 530 nm and emission at 610 nm) on a 0.2-µm pore size polycarbonate filter previously stained with Irgalan black (Hobbie et al. 1977). Epifluorescence microscopic counts of total bacteria were done using a Zeiss SL-16

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Table 1. Physical chemical parameters and community metabolism estimates from changes in DO and CO_2 and uptake of [^{14}C]bicarbonate.

	Run 1 (cylindrical)			Runs 2 and	Run 2 (cylindrical)		Run 3 (rectangular)	
	Overall	Dark	Light	3 overall	Dark	Light	Dark	Light
Current velocity ($cm \cdot s^{-1}$)								
In situ (5 mm over sediment)	23.9±20.5							
In situ (1 mm over sediment)				2.7±0.6				
In chamber (1 mm over sediment)		0.9	0.9		1.7	1.7	2.4	1.7
Organic matter (%)		4.35	5.90		7.35	6.48	7.04	6.19
Bacteria·cm^{-2} ($\times 10^8$)		6.60	6.67		7.91	7.61	7.13	5.93
Chlorophyll *a* ($mg \cdot m^{-2}$)		354	922		963	710	1286	1064
Water temperature (°C)	20–23			20–23				
Total alkalinity ($mg \cdot L^{-1}$)	157.5±5.0			164.0±5.0				
$NO_2 + NO_3$ ($\mu g \cdot L^{-1}$)	137.9±1.5			138.8±3.7				
NH_4 ($\mu g \cdot L^{-1}$)	109.4±7.7			339.3±9.6				
SRP ($\mu g \cdot L^{-1}$)	5.4±4.5			nd				
^{14}C uptake ($\mu g\ C \cdot cm^{-2} \cdot h^{-1}$)		2.07	23.34		1.50	30.55	2.65	15.69
DO change ($mg \cdot L^{-1} \cdot h^{-1}$)		–1.01	–0.04		–0.97	–0.10	–0.48	0.21
CO_2 change ($\mu mol \cdot L^{-1} \cdot h^{-1}$)		4.54	–0.17		3.86	–0.04	4.44	–0.60

Note: Run 1 was performed on June 9, and runs 2 and 3 were performed on June 10. nd, not detected.

microscope with Zeiss filter set No. 487714. Sample dry weights were obtained as described above.

Calculation of metabolic parameters and conversion factors

Chlorophyll *a* concentrations were converted to algal carbon concentrations using a factor of 30, suitable for communities in nutrient-rich, unshaded environments (Vollenweider 1974; Sumner and McIntire 1982). Total biomass C was estimated from AFDM determinations assuming a 50% carbon content.

In experiment 1, carbon fixation was estimated from ^{14}C uptake after Vollenweider (1974) with correction for excreted and adsorbed carbon. DO fluxes were converted to carbon equivalents using a respiratory quotient of 0.85 and a photosynthetic quotient of 1.2 (Vollenweider 1974), and gross primary productivity (GPP) was calculated by adding the change in the dark chamber to the change in the light chamber. GPP was directly calculated from CO_2 change in a similar manner.

In experiment 2, a diel rate of change curve was constructed for each chamber using data from 0 to 24 h. The hourly rate of DO change (ΔDO) was determined by subtracting the DO concentration at $t - 60$ min from the value at t min for each 10-min reading and averaging the six ΔDO values for a given hour. Use of the average value minimized any effect of instrumentation noise on measurement of the metabolic signal. Estimates of daily community respiration (CR_{24}) were obtained by applying DO change measured during darkness to the 24-h period. GPP was determined by integrating changes in DO during daylight hours and adding community respiration for the same period. The time of sunrise and sunset were taken as the time when the radiometer indicated first and last light, with darkness defined as incident solar radiation levels <0.1% of midday irradiance. Calculation of net daily metabolism ($GPP - CR_{24}$) and the GPP/CR_{24} ratio allowed determination of the predominant metabolic process for the diel period.

Results

Community composition

Antecedent hydraulic conditions at the site were sufficiently stable, and light transmission adequate, to permit development of a cohesive periphyton mat at the water–sediment interface in the soft-bottom edgewater habitat. Bacterial densities in epipelic communities used in experiment 1 averaged $6.98 \times 10^8 \pm 0.72 \times 10^8$ cells·cm^{-2} ($1.19 \times 10^{10} \pm 0.24 \times 10^{10}$ cells·g dry sediment^{-1}; mean ± SD, $n = 6$), and densities overlapped between chambers and days (Table 1). Chlorophyll *a* concentrations ranged from 700 to 1300 $mg \cdot m^{-2}$ (989 ± 210 $mg \cdot m^{-2}$, $n = 5$), except for the community placed in the dark chamber in run 1, which had only 354 $mg \cdot m^{-2}$ (Table 1). Confocal microscopic examination of the epipelic community showed an abundance of filamentous cyanobacteria, particularly *Oscillatoria* sp.; unicellular forms, especially diatoms, were conspicuously rare. Examination of the mat at 100-µm intervals indicated maximal algal biomass in the upper 300 µm and a sharp decrease below 500 µm (Fig. 1).

Epilithic communities used in experiment 2 were dominated by the filamentous chlorophyte *Cladophora glomerata*, with the diatoms *Cocconeis* sp. (attached to the *Cladophora glomerata*), *Epithemia* sp., *Cymbella* sp., and cyanobacteria (*Oscillatoria* sp. and *Phormidium* sp.) also abundant. *Suriella* sp., *Navicula* sp., and *Scenedesmus* sp. were also present, along with other rarer unidentified taxa. Chlorophyll *a* concentrations were lower than on sediments, ranging from 47 to 150 $mg \cdot m^{-2}$ (Table 2). Total organic matter was high, however, and algal biomass contributed only $6.06 \pm 1.16\%$ ($n = 8$) of the AFDM on the rocks, which emphasizes the heterotrophic nature of the community.

Experiment 1: comparison of primary productivity measured by DO and CO_2 change and ^{14}C uptake

Current velocities in the chambers in runs 1, 2, and 3 were 33, 63, and 76%, respectively, of that 1 mm over the river bed (Table 1). Organic matter percentages and bacterial densities varied less than twofold between chambers, but benthic chlorophyll *a* concentration varied approximately fourfold. Water temperature, total alkalinity, and $NO_2 + NO_3$ concentrations were similar on each day, but NH_4 and SRP concentrations varied considerably.

Community respiration rates exceeded O_2 production in the cylindrical chambers in runs 1 and 2, resulting in a negative O_2 flux even in the light (Table 1). In each run, GPP expressed on an areal basis was greatest from ^{14}C incorporation, intermediate from DO change, and least from CO_2 change (Fig. 2). The pattern held when data were normalized by chlorophyll,

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Fig. 1. Community development (revealed by autofluorescence of chlorophyll *a*) at 100 µm depths through a cyanobacterial mat overlying sediments.

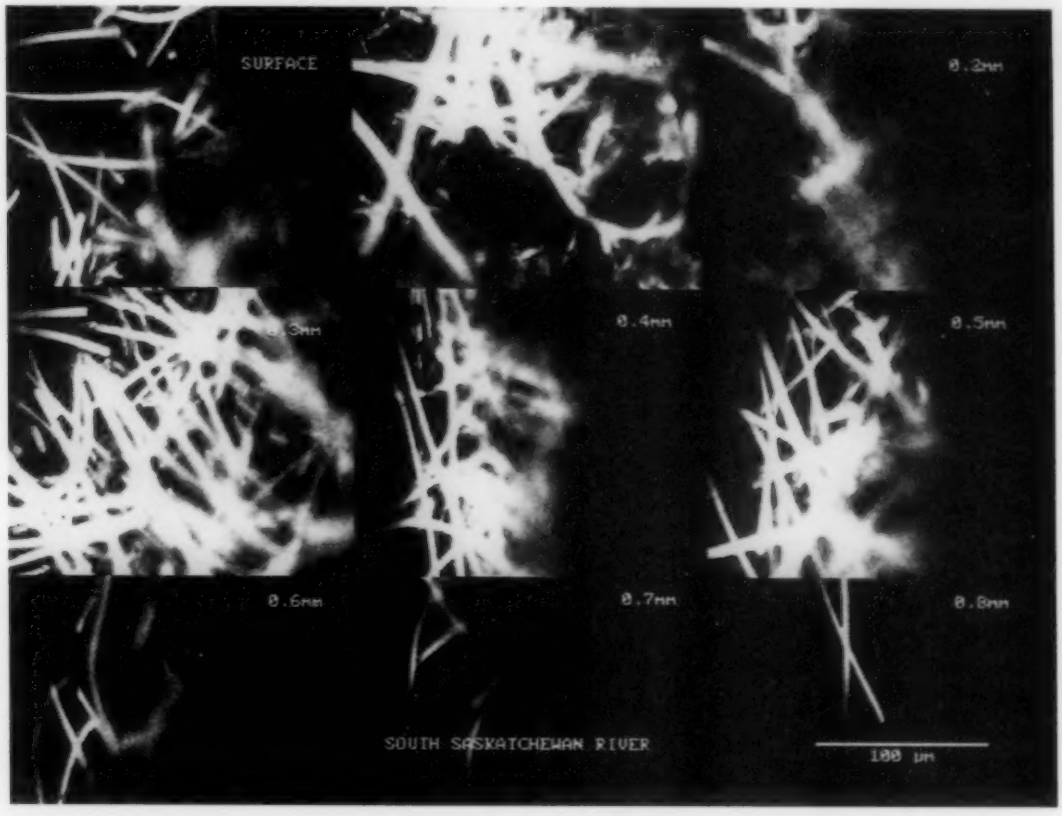

Table 2. Community metabolic parameters at different velocities in chambers.

	Run 1 (June 11–12)[a]				Run 2 (June 12–13)[b]			
	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Chamber 2	Chamber 3	Chamber 4	Chamber 5
River current 1 mm above rock ($cm \cdot s^{-1}$)	7.6	5.8	6.6	7.1	>6.5	>6.5	>6.5	>6.5
River current at 0.6 depth ($cm \cdot s^{-1}$)	74	70	71	71	31	38	41	13
Chamber current 1mm above rock ($cm \cdot s^{-1}$)	3.4	0.8	6.5	>6.5	0.24	0.20	2.90	2.30
Chlorophyll *a* ($mg \cdot m^{-2}$)	72	47	50	126	76	107	57	150
Algal C ($mg \cdot m^{-2}$)	2148	1411	1508	3787	2273	3200	1697	4507
AFDM ($mg\ C \cdot m^{-2}$)	34 992	18 409	29 787	65 942	36 500	45 833	42 950	67 124
Algal C/total organic C (%)	6.14	7.67	5.06	5.74	6.23	6.98	3.95	6.71
Metabolic activity								
GPP ($g\ DO \cdot m^{-2} \cdot day^{-1}$)	8.11	4.70	4.51	14.58	14.48	20.20	8.83	15.33
CR_{24} ($g\ DO \cdot m^{-2} \cdot day^{-1}$)	7.00	3.36	3.96	11.89	11.30	15.92	6.71	12.58
NDM ($g\ DO \cdot m^{-2} \cdot day^{-1}$)	1.11	1.34	0.55	2.69	3.18	4.28	2.12	2.75
GPP/CR24	1.16	1.40	1.14	1.23	1.28	1.27	1.32	1.22
$g\ DO \cdot mg\ Chl\ a^{-1} \cdot day^{-1}$	0.113	0.100	0.090	0.115	0.191	0.189	0.156	0.102

[a]Chemical parameters at start of run 1 (mean ± SD, $n = 2$): 184 ± 5 µg $NH_4 \cdot L^{-1}$, 122 ± 5 µg $NO_2 + NO_3 \cdot L^{-1}$.
[b]Chemical parameters at start of run 2 (mean ± SD, $n = 2$): 269 ± 0.6 µg $NH_4 \cdot L^{-1}$, 192 ± 5 µg $NO_3 + NO_2 \cdot L^{-1}$, 1.7 ± 0.5 µg $SRP \cdot L^{-1}$.

except for run 1 where a large difference in chlorophyll content between chambers resulted in greater chlorophyll-normalized corrections for the dark incubation (subtracted for the ^{14}C technique and added for the gas change method).

Areal productivity estimates were similar (within 20%) between days in the cylindrical chambers for each method despite daily variation in water velocity and concentrations of NH_4 and SRP (Fig. 2). However, chlorophyll-specific GPP differed by factors of 2.1, 3.3, and 5.0 for the ^{14}C, DO, and CO_2 methods, respectively, in runs 1 and 2. Comparison of

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Fig. 2. Estimates of GPP from simultaneous measurements of ^{14}C incorporation, DO change, and CO_2 change using cylindrical chambers on June 9 (run 1) and June 10 (run 2) and rectangular chambers on June 10 (run 3).

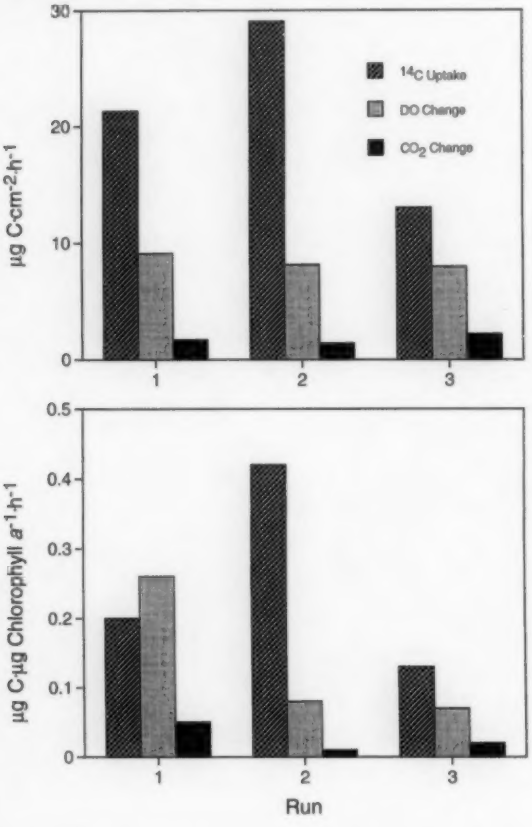
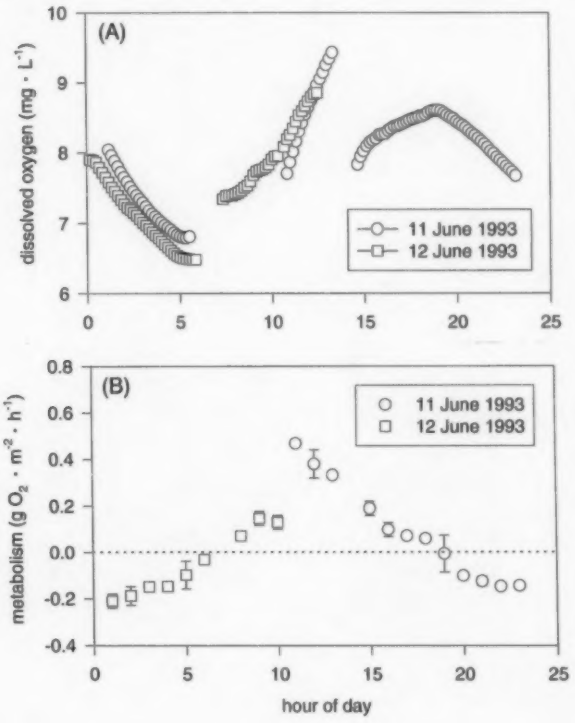


Fig. 3. (A) DO concentration logged at 10-min intervals for chamber 4 during run 2 on June 11–12, 1993. Daylight period is 04:30–21:50. The breaks in the DO record are associated with chamber flushing for sample collection. The chamber was filled with subsaturated water at 14:00 on June 11. (B) DO metabolism for chamber 4, expressed as an hourly rate of change curve calculated from data shown in A. Error bars are ±1 SD, with $n = 6$ for each hour for the period 11:00 on June 11 through 11:00 on June 12.



chambers of different design (runs 2 and 3) showed that the areal ^{14}C estimate was twofold greater in the cylindrical than in the rectangular chambers, although there was little difference for the gas change methods (Fig. 2). Chlorophyll-specific GPP differed between chambers by factors of 3.2, 1.1, and 2.0 for the ^{14}C, DO, and CO_2 methods, respectively.

DO changes in the individual chambers were significantly negatively correlated with CO_2 changes (Pearson correlation coefficient, $r = -0.907$, $p < 0.05$, $n = 6$). ^{14}C uptake was significantly correlated with CO_2 change ($r = -0.891$, $p < 0.05$, $n = 6$) and marginally with DO change ($r = 0.776$, $p = 0.07$, $n = 6$). The proportion of photosynthate excreted was slightly higher in the cylindrical chambers (32 and 39% in runs 1 and 2, respectively) than in the rectangular chamber (26% in run 3).

Experiment 2: effect of water velocity on epilithic metabolism

The weather between midday June 11 and June 13 was predominantly overcast and rainy, with PAR decreasing from 16 (run 1) to 7 mol·m^{-2}·day^{-1} (run 2). Initial nutrient concentrations were higher for run 2 of this experiment than run 1 and corresponded to elevated river discharge. In run 1 the water velocity in each chamber was set to a value between 0.8 and >6.5 cm·s^{-1} (measured 1 mm above rocks with a thermistor flow meter), a range that encompassed the near-bottom current velocities where the rocks were collected (5.8–7.6 cm·s^{-1}). Data for the highest velocity in this run were questionable; thus, they are reported as >6.5 cm·s^{-1}, which was the next lowest velocity measured with confidence. In run 2, duplicate chambers were set at either low (~0.22 cm·s^{-1}) or high (2.6 cm·s^{-1}) velocity (Table 2).

DO changes during predawn hours on June 11 and 12 were strikingly similar (Fig. 3A). GPP slightly exceeded CR_{24} in all chambers in each run, yielding a positive value for net daily metabolism and a GPP/CR_{24} ratio slightly in excess of 1.0 despite the strongly heterotrophic nature of the communities (Table 2). GPP normalized for chlorophyll *a* was similar in all chambers in run 1 (0.09–0.12 g DO·mg chlorophyll a^{-1}·day^{-1}, but the spread in the data was slightly greater in run 2 (0.10–0.19 g DO·mg chlorophyll a^{-1}·day^{-1}).

Comparison of metabolic data for each chamber with environmental parameters and experimental water velocity showed that areal GPP and CR_{24} were significantly correlated with

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Table 3. Changes in nutrient concentrations over four time periods during runs of the current velocity experiment.

Period	NH_4 ($\mu g \cdot L^{-1}$) Start	Finish	ΔNH_4 ($\mu g \cdot L^{-1} \cdot h^{-1}$)	$NO_2 + NO_3$ ($\mu g \cdot L^{-1}$) Start	Finish	$\Delta NO_2 + NO_3$ ($\mu g \cdot L^{-1} \cdot h^{-1}$)
Dark						
Run 1: 23:45–06:00	184±5	272±18	14	122±5	174±17	8
Run 2: 00:45–05:00	269±1	239±3	–6	192±5	167±15	–5
Light						
Run 1: 10:00–13:15	272±8	174±35	–30	140±8	158±10	5
Run 2: 06:00–15:50	221±4	8±7	–22	187±26	155±13	–3

Note: Values are given as the mean ± SD ($n = 7$ or 8).

chlorophyll *a* concentration (Spearman rank correlation coefficients, $r = 0.881$, $p < 0.01$; $r = 0.929$, $p < 0.001$, respectively, $n = 8$) but not with water velocity ($p > 0.05$).

Changes in nutrient concentrations in the chambers were measured between midnight and dawn and during midday hours during each run. Mean NH_4 concentrations either increased or slightly decreased during darkness but decreased at a greater rate in the light (Table 3). Final NH_4 concentrations at the end of the ~10-h incubation period in run 2 averaged only 8 $\mu g \cdot L^{-1}$ or 4% of the initial concentration. $NO_3 + NO_2$ concentrations varied less than NH_4 concentrations over the diel period whereas SRP concentrations were usually below the detection limit of 3 $\mu g \cdot L^{-1}$.

During light incubations, increases in DO in the eight individual chambers ranged from 0.33 to 1.18 $mg \cdot L^{-1} \cdot h^{-1}$ and decreases in NH_4 from 20.09 to 38.92 $\mu g \cdot L^{-1} \cdot h^{-1}$, while changes during darkness ranged from –0.13 to –0.73 mg $DO \cdot L^{-1} \cdot h^{-1}$ and from –7.92 to 14.48 μg $NH_4 \cdot L^{-1} \cdot h^{-1}$. Hourly changes in DO normalized for area, chlorophyll *a* content, and AFDM were all significantly correlated positively with PAR and negatively with change in NH_4 concentration for the dark and light periods but not with current velocity and change in $NO_2 + NO_3$ (Table 4). Changes in NH_4 and $NO_2 + NO_3$ concentrations were significantly negatively correlated during the light incubation periods ($r = -0.821$, $p < 0.05$, $n = 7$ chambers).

Table 4. Results of Spearman rank correlations showing relationships (r values) between hourly rates of change in oxygen and nitrogen, chamber flow velocity, and light during four time periods in flow velocity experiments.

	n	$\Delta DO \cdot m^{-2}$ ($n = 15$)	$\Delta DO \cdot Chl\ a^{-1}$ ($n = 15$)	$\Delta DO \cdot AFDM^{-1}$ ($n = 15$)
Velocity	15	0.068	0.061	0.021
$\Delta NO_2 + NO_3$	13	0.098	0.165	0.165
ΔNH_4	14	–0.811***	–0.711**	–0.626*
Cumulative PAR[a]	15	0.796***	0.839***	0.821***

Note: Chl *a*, chlorophyll *a*; *n*, number of estimates. The smaller of the two *n* values was used for statistical comparisons. Significance levels: *, $p = 0.05$; **, $p = 0.01$; ***, $p = 0.001$.

[a]Photosynthetically available radiation accumulated during incubation period.

Experiment 3: comparison of metabolism estimates from microelectrode measurements and macroscale DO changes

Characterization of the depth of biological activity in the epipelic mat with O_2 microelectrodes showed maximal activity in the top 0.5–2.5 mm (Figs. 4A and 4B). This zone exhibited supersaturated DO concentrations in the light. Within 5 mm, respiration exceeded photosynthesis and the system became completely anoxic. Measurements of DO production, particularly net photosynthesis, made with microelectrodes yielded rates two to four times higher than those measured from bulk-water DO change (Table 5).

Water velocity profiles showed considerable variation near the sediment surface owing to turbulence, with velocities of 2.3 $cm \cdot s^{-1}$ at 1 cm above the surface corresponding to 1.7 $cm \cdot s^{-1}$ at 1 mm above the surface (Fig. 4C). This variability and sharp increase in velocity suggest that significant diffusion is possible at the surface of the periphyton mat.

Profiles of DO measured with a microelectrode through a mass of *Cladophora* sp. that extended 2 mm above a rock showed that concentrations were significantly affected by water velocity (Fig. 4D). At 0 $cm \cdot s^{-1}$, DO was supersaturated up to the surface of the mat. At elevated water velocity (0.7 $cm \cdot s^{-1}$), DO concentrations were above saturation but only at the bottom of the mat. At slightly higher water velocities (1.09 and 1.46 $cm \cdot s^{-1}$), DO concentrations were constant throughout and 1 mm above the mat, and zones of supersaturated O_2 were completely removed. Thus, transport of metabolic gases was high relative to production at all but the lowest water velocities. These measurements were made under lower light intensities than the productivity measurements shown in Figs. 4A and 4B.

Discussion

Community metabolism: comparison of macroscale methods

We anticipated that estimates of GPP would be higher from the gas change methods than from ^{14}C uptake because the former include respiration of the entire community and the latter reportedly measure something between net and gross primary productivity (Vollenweider 1974). The opposite occurred (Fig. 2), lending support to the claim that short incubation periods provide ^{14}C estimates closer to GPP (Dring and Jewson 1982). Hunding and Hargrave (1973) also reported that ^{14}C uptake yielded an estimate of productivity that was two times higher than that from gas change, especially under high light intensities. However, Bott and Ritter (1981) found that in slightly longer incubations (4 h) estimates from ^{14}C uptake and DO change agreed within a factor of two, with virtually identical results in four of seven experiments. Even so, the ^{14}C procedure could underestimate primary productivity because the specific activity of ^{14}C in the periphyton mat and underlying sediments can be lower than in the overlying water (Revsbech et al. 1981). However, if our ^{14}C data are underestimates, then the difference between that method and gas change techniques

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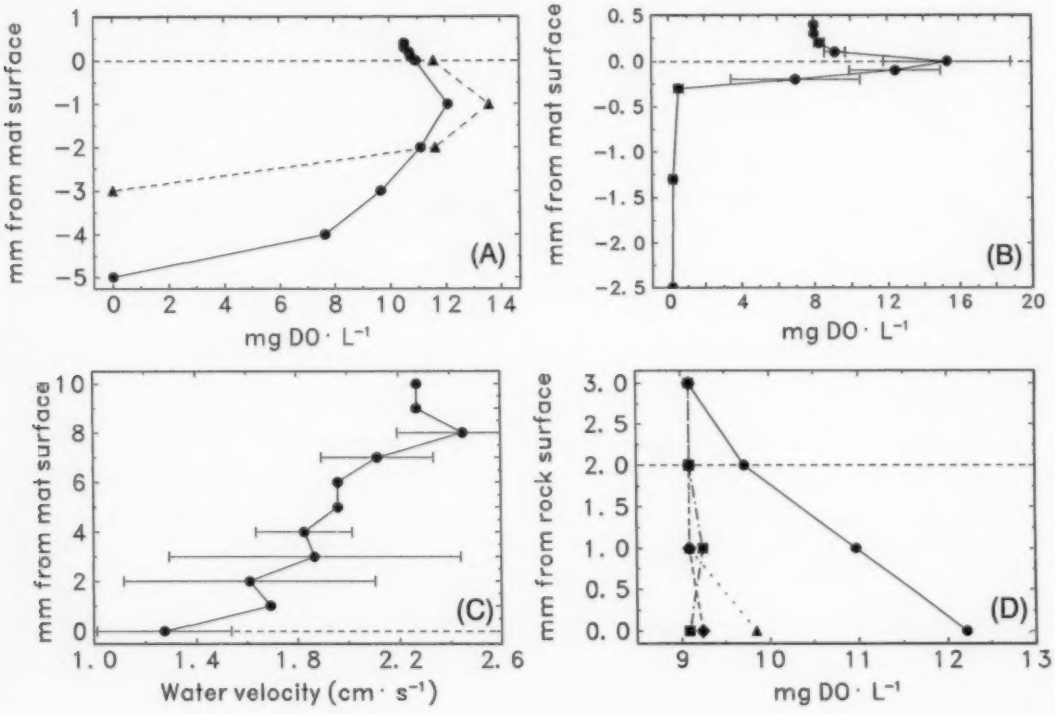
Fig. 4. (A) Two profiles of DO concentration taken with a microelectrode, 1 cm apart, in sediment with a community dominated by *Oscillatoria* sp. on the surface (June 9, 1993, 16:53, under bright sun). Mat surface is shown with a broken horizontal line. (B) Composite of two DO microelectrode profiles through a periphyton mat on sediments (June 10, 1993, 11:20, under bright sun). Mat surface is shown with a broken horizontal line. Error bars are ±1 SD. (C) Current velocity profile 1 cm above periphyton mat. Profile represents mean of two profiles taken in the same location over a 15-min period. Error bars are ±1 SD. Mat surface is shown with a broken horizontal line. (D) DO profiles above a rock colonized with *Cladophora* sp. for various water velocities ($cm \cdot s^{-1}$): ●, 0; ▲, 0.7; ■, 1.09; ◆, 1.46. Water velocity was measured 3 mm above the rock; the *Cladophora* mass extended 2 mm above the rock, shown by the broken horizontal line (June 11, 1993, under cloudy conditions).



would be even greater. Turner et al. (1983) reported agreement between ^{14}C uptake and changes in dissolved inorganic carbon (DIC), but estimates from these methods were the most different in our study.

The discrepancy in our ^{14}C and gas change measurements could possibly be related to diel rhythms of CO_2 fixation and O_2 release. Our incubations were conducted during the early to middle afternoon on both days. Gallegos et al. (1983) reported that O_2 production is often reduced in the afternoon in dense algal cultures. Our thick periphyton mats might have been entering a phase of low O_2 production at the start of our incubations. While differences between the ^{14}C and DO techniques could also result from O_2 bubble formation (Revsbech et al. 1981) with a resulting underestimate of DO change, we attempted to prevent that by stopping incubation before O_2 bubbles were observed on the chamber walls.

Water velocity experiment

Although differences between water velocities in situ and those in chambers have been cause for concern, our epilithon production rates were not significantly correlated with chamber current velocities over the range 0.2 to >6.5 $cm \cdot s^{-1}$ (Table 2). However, they were positively correlated with chlorophyll *a* concentrations. Thus, although replication was not extensive in our experiments, epilithic biomass affected metabolic rates, but variation in current velocity in the range studied had little effect.

Several studies have documented that increased flow enhanced rates of mass transfer between the organism and the environment and enhanced metabolic activity (Whitford and Schumacher 1961; Horner and Welch 1981; Dodds 1989). Hickey (1988*a*), working with sewage fungus biofilms from several rivers, reported an approximately linear relationship between O_2 flux and velocity over the range of 5–25 $cm \cdot s^{-1}$. In contrast, we found that metabolism was not strongly affected by water velocity when in a lower range. Borchardt (1994) recently showed that the maximal photosynthetic rates of *Spirogyra fluviatilis* occurred at an intermediate velocity (12 $cm \cdot s^{-1}$), rather than the maximum, over the range of 3–30 $cm \cdot s^{-1}$. Others have noted that variability in metabolic rate was more a function of community and microhabitat characteristics than chamber design, flow rate, and measurement technique (Hall et al. 1979). Turbulence characteristics generated by features of the sediment or biofilm surface interacting with water velocity in the chamber may be more important than the actual velocity imposed. Patterson et al. (1991), working

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Table 5. O_2 microelectrode and whole chamber estimates of photosynthesis.

		Microelectrode				Chamber DO change			
Date	Time	GPP	*n*	Net diffusion	*n*	GPP	*n*	NPP	*n*
9 June	15:52–16:43	0.061±0.006	2	0.0059±0.0070	3	0.030	1	–0.001	1
10 June	11:20	0.054±0.017	3	0.0268±0.0258	2				
10 June	15:27	0.082±0.004	2	0.0304	1	0.022±0.007	3	0.0023±0.0056	3

Note: All estimates are given as the mean ± SD, in mg $O_2 \cdot cm^{-2} \cdot h^{-1}$. Gross O_2 microelectrode photosynthesis was calculated as outlined in Revsbech and Jorgensen (1986). Net microdiffusion was calculated from the steepness of the diffusion boundary. Measurements on June 9 and June 10 at 11:20 were made in the river on undisturbed sediments. Measurements on June 10 at 15:27 were made in a chamber with current velocity matched to that above the collected sediment. GPP, gross primary production; NPP, net primary production.

with marine corals, stressed the importance of turbulence in increasing photosynthesis and respiration rates. Hickey (1988*b*) also emphasized the need to consider turbulence characteristics and match the boundary velocity in the river and the chamber when trying to replicate field conditions in chambers.

Algae are reported to prefer NH_4 over NO_3 as a source of inorganic N (Dodds and Priscu 1989). The significant correlation between changes in concentrations of DO and NH_4 and the higher rates of NH_4 uptake in the light compared with the dark indicate that this was probably true for our communities (Table 3). However, algal biomass comprised only 5.1–7.7% of epilithic total organic matter and NH_4 also would be used by heterotrophic organisms, especially nitrifying bacteria that were undoubtedly present in this nitrogen-rich system. The negative correlation between changes in NH_4 and $NO_2 + NO_3$ supports nitrification activity. Tissue concentrations of N (21.0–28.5 mg N·g $AFDM^{-1}$) and P (3.1–3.9 mg P·g $AFDM^{-1}$) for our periphyton communities were in the range of those reported for *Spirogyra fluviatilis* (Borchardt 1994), *Cladophera glomerata*, and *Stigeoclonium tenue* (Rosemarin 1982).

As a general rule, NH_4 and NO_3 concentrations of 50 $\mu g \cdot L^{-1}$ (approximately twice the half-saturation constants for these nutrients) are sufficient to support sustained phytoplankton photosynthesis (U.S. Environmental Protection Agency 1985), and these levels also apply to periphyton communities in the Truckee River, Nevada (J.T. Brock, unpublished data). The final $NO_2 + NO_3$ concentrations in the chambers following incubation periods of ~3–10 h do not suggest NO_3 limitation of photosynthesis in our experiments. Given the N-rich water chemistry at our site and the frequency with which we changed chamber water, it is unlikely that NH_4 became limiting in our experiments, with one exception (the light incubation in which the NH_4 concentration reached 8 $\mu g \cdot L^{-1}$).

SRP levels in river water were too low to allow detection of P uptake from concentration changes. Furthermore, Paul et al. (1989) noted that orthophosphate uptake was predominantly by seston rather than epibenthic biomass. We did not measure sestonic biomass in our systems, but competition with the benthos for P could have been significant, since the river was turbid. An SRP concentration of 10 $\mu g \cdot L^{-1}$ is usually considered sufficient to sustain photosynthesis (U.S. Environmental Protection Agency 1985; Bothwell 1989). Given the low SRP concentrations in the South Saskatchewan River, P was probably a limiting macronutrient for our communities. Processes leading to nutrient replenishment occur in chambers, but our results highlight the need to monitor nutrient concentrations during chamber metabolism measurements.

The extent to which river velocities and turbulence characteristics should be reproduced when using metabolism chambers needs further study. Elevated water velocities require substantial circulation capabilities (and electrical power requirements) that can be difficult to obtain when working in the field. As circulation flow increases within a closed chamber, so does the pressure (which can also affect DO probe response) and the heat generated by fluid motion. Further, the physical size of the system required to properly scale boundary flow conditions to the highly turbulent conditions found in some river habitats is impossible to achieve in a portable device. Although means are available to measure velocity profiles and adjust turbulence characteristics within chambers (Lacoursière and Craig 1990), such tailoring of flow can require considerable time and effort. Some benthic communities may have a more sensitive physiological response to flow conditions than others. For example, our results, although preliminary, at flow velocities of 0.002–0.06 $m \cdot s^{-1}$ suggest there was a threshold above which current velocity in the chamber was of little consequence to metabolism of the rock and sediment communities studied. If so, determination of that threshold velocity for different periphyton communities would be useful because excessive circulation conditions could be avoided. We suspect that the response of the periphyton community to water velocity and turbulence could vary considerably with the composition, stage of development, and physiological condition of the biota.

Comparison of microelectrode measurements with macroscale DO readings

The higher rates of DO production measured with microelectrodes compared with bulk-water changes (Table 5) are partially attributable to the fact that the microelectrode method requires that equilibrium concentrations of O_2 be established before measurements are initiated. This requires a high-intensity and stable light regime, which in the field means full sunlight without clouds. In contrast, chamber estimates can be made under all light conditions. In addition, since the microelectrode can be precisely positioned, the tendency is to select areas with an obvious mat. This probably results in an overestimatation of areal rates of productivity because the sediment samples used in chamber experiments were larger and incorporated a wider range of mat characteristics. Finally, Glud et al. (1994) showed that the electrode tip can artificially compress the diffusion boundary, an artifact that would lead to overestimation of productivity. Interestingly, the microelectrode production estimates for the South Saskatchewan River were more like those from ^{14}C uptake than those from the macroscale DO method.

Measurements of DO concentration as a function of water

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velocity in *Cladophora* mats indicated that very little flow was required to remove supersaturated O_2 from the mat (Fig. 4). This is in direct contrast to previous findings (Dodds 1991*a*) that elevated DO concentrations within a *Cladophora* mat were maintained even with water velocities up to 40 $cm \cdot s^{-1}$ immediately outside the mat. The discrepancy may relate to the size and productivity of the mat, since the earlier study was undertaken with a larger algal mass under higher irradiance. The larger mass may isolate the external flow and thus be an important determinant of microzone formation. Moreover, the tendency of large masses to compress under higher water velocities (Dodds and Gudder 1992) may accentuate differences in DO gradients between the smaller tufts used in this study and the larger ones used previously. Since supersaturation of O_2 can indicate CO_2 limitation (Raven 1992) and water movement can stimulate nutrient uptake (Whitford and Schumacher 1961; Horner and Welch 1981) and photosynthesis of *Cladophora* sp. (Dodds 1991*b*) as well as other periphyton species (Dodds 1989), the conditions requiring careful attention to water velocity still need further elucidation.

Physical disruption may be a problem when removing sediment samples for measurements with microelectrodes. The steep O_2 gradients we observed are typical (Revsbech et al. 1981; Revsbech and Jorgensen 1986) and indicate little disruption to the community during removal. That the anoxic zone was so close to the surface in the sediment samples is probably related to high light attenuation typical of active periphyton communities, and concomitant decreases in photosynthesis with depth (Dodds 1992). The steep velocity profiles immediately above the sediments indicate that significant turbulent transport may occur at edges and other disrupted locations of sediment cores. Disruption of the sediments, or transport of water through even very shallow portions of the sediment, may bring anoxic water into the chamber and lead to lower estimates of primary production than actually occur.

The confocal microscopy results were consistent with the microelectrode determination of zones of maximal microbial activity. The zonation of algal biomass was roughly consistent with zones of higher oxygen production (Figs. 1 and 4). Observations of the algal mats using SCLM indicated that imaging of undisturbed cores could be achieved to depths of a few hundred micrometres when a water-immersion lens (40×, 0.55 NA) was used rather than the high NA 1.4 oil-immersion 60 or 63× objectives typically recommended for SCLM. The use of a water-immersion lens offered the maximal working distance while allowing optical sectioning, but mechanical sectioning was required to facilitate imaging of all mat material. Such images provide a basis for estimation of algal and bacterial biomass with depth, on the basis of image processing and analysis and calculation of percentage area or total area of algal filaments (cells) within each image, as has been done for bacteria in other studies (Lawrence et al. 1991; Wolfaardt et al. 1994).

In conclusion, this study explored several questions concerning the use of chambers for metabolism measurements. While additional work with greater replication is still required, our comparison of methods showed that ^{14}C uptake yielded higher estimates of GPP than either the DO or CO_2 change methods, lending support to the contention that the ^{14}C uptake procedure measures GPP in short-term incubations. Secondly, measurements made with oxygen microelectrodes at the highest level of spatial resolution provided the greatest estimates of primary productivity, perhaps because measurements were made with active communities under optimal conditions for photosynthesis. Thirdly, our water velocity experiment indicated that GPP was affected more by the biomass of algae introduced into the chamber than the water velocity in the chamber over the range 0.2 to ~6.5 $cm \cdot s^{-1}$. Those runs showed that it is necessary to pay attention to the possibility of nutrient limitation when conducting chamber studies, especially in nutrient-poor environments. Finally, the method used to measure the activity of the benthic communities produced greater variability in metabolic rate estimates than either the chamber's physical configuration or internal flow dynamics.

Our results reinforce earlier findings (Bott et al. 1978) regarding the difficulty of comparing estimates of benthic community metabolism obtained with different apparatus and analytical procedures. One should select the methods most consistent with the experimental objectives, which can range from analyses of microscale processes to estimations of ecosystem metabolism. Furthermore, metabolism data should not be collected in a routine manner without assessing potential sources of error.

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REVIEW / SYNTHÈSE

A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes

Enric Cortés

Abstract: Using real data sets of elasmobranch fishes as examples, this paper presents a critical review of selected methods and statistical approaches used in fish feeding studies and makes recommendations on the application of such methodology. The percent index of relative importance is proposed as a standardized measure in dietary analyses, and a three-dimensional graphical representation of the diet is introduced. Multiway contingency table (log–linear) analysis is recommended to test for dietary variations. Caution is advised when using rank correlation to study dietary overlap and parametric tests when stomach content data do not satisfy parametric assumptions. Sampling gear type, experimental design, and statistical tests can affect results on diel feeding chronology, and stomach content weights do not suffice to interpret diel feeding chronology. On the basis of sampling requirements and model assumptions, the Diana and Olson–Mullen methods appear to be the most appropriate approaches for estimating daily ration in sharks. Use of resampling techniques is highly desirable because they provide a measure of the error in daily ration estimates. Using several criteria to evaluate the best-fitting model of gastric evacuation in fishes is also strongly advocated. Overall, increased consolidation of methods and analyses is recommended to facilitate comparative studies.

Résumé : En prenant pour exemples des séries de données réelles sur des poissons élasmobranches, nous présentons une analyse critique de certaines méthodes et approches statistiques utilisées dans les études sur l'alimentation des poissons, et nous faisons des recommandations sur l'application de cette méthodologie. Nous proposons un indice de l'importance relative en pourcentage comme mesure normalisée pour les analyses alimentaires, et nous introduisons une représentation graphique tridimensionnelle du régime alimentaire. L'analyse (log–linéaire) de tableaux multidimensionnels est recommandée pour étudier les variations dans l'alimentation. Nous conseillons la prudence dans l'emploi de la corrélation de rangs pour étudier les chevauchements dans le régime alimentaire et des tests paramétriques quand les données sur le contenu stomacal ne satisfont pas les hypothèses paramétriques. Le type d'engin utilisé pour l'échantillonnage, le protocole expérimental et les tests statistiques peuvent avoir un effet sur les résultats de la chronologie de l'alimentation nycthémérale, et le poids du contenu stomacal ne suffit pas à interpréter cette chronologie. D'après les besoins de l'échantillonnage et les hypothèses des modèles, les méthodes de Diana et d'Olsen–Mullen semblent être les plus appropriées pour estimer la ration quotidienne chez les requins. L'emploi de techniques de rééchantillonnage est très souhaitable car cette démarche donne une mesure de l'erreur dans les estimations de la ration quotidienne. Nous recommandons aussi fortement d'avoir recours à plusieurs critères pour évaluer le modèle qui offre le meilleur ajustement pour l'évacuation gastrique chez les poissons. Globalement, il est recommandé de regrouper de plus en plus les méthodes et les analyses pour faciliter les études comparatives.
[Traduit par la Rédaction]

Introduction

The study of diets and food habits of fish and other marine vertebrates through the examination of stomach contents has become standard practice (Hyslop 1980). However, a review of dietary, food habit, and food consumption studies of fish reveals a lack of consistent methodological approaches and application of statistical tests to analyze results. The present study was prompted by a perceived need to promote consistency, facilitate comparisons, and verify the proper application of methodological approaches in feeding studies.

The purpose of this study is to examine some selected methods and statistical approaches commonly used in diet, food habit, and food consumption studies of fish and, with the help of illustrative examples, present recommendations on how to obtain, analyze, and report data. Specifically, the objectives are to (*i*) review the most commonly used indices of dietary importance and propose the use of a standardized index and a new graphical method to illustrate prey importance and predator feeding strategy and homogeneity at the population level; (*ii*) reemphasize the need for reporting precision and determining sample size sufficiency in dietary descriptions; (*iii*) review the use of multivariate statistics, log–linear analysis, and multiway contingency table analysis to detect seasonal, ontogenetic,

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Table 1. Relative importance of food type by different indices for two species of sharks.

Food type	%N	%W	%O	IRI	%IRI
Lemon shark (*Negaprion brevirostris*)					
Fishes	75.4	98.4	131.2 (86)	22 802 (14 947)	97.2 (96.6)
Molluscs	1.5	0.9	3.1 (3.1)	7 (7)	0.03 (0.05)
Crustaceans	7.7	0.4	14.1 (12.5)	114 (101)	0.5 (0.6)
Bonnethead shark (*Sphyrna tiburo*)					
Crustaceans	63.4	82.9	97.4 (88.9)	14 250 (13 006)	82.2 (84.9)
Molluscs	0.8	0.8	3.5 (2.3)	6 (4)	0.03 (<0.01)
Teleosts	0.5	0.7	1.5 (1.5)	2 (2)	0.01 (<0.01)
Angiosperms	26.7	7.0	78.4 (56)	2 642 (1 888)	15.2 (12.4)
Miscellaneous	8.6	8.6	25.4 (24.8)	437 (427)	2.5 (2.8)

Note: %N, percent by number; %W, percent by weight; %O, percent frequency of occurrence; IRI, index of relative importance; %IRI, percent index of relative importance. Values for %O in parentheses are observed nonadded values (i.e., corresponding to a particular food type, not the sum of its components). Values for IRI and %IRI in parentheses were calculated using observed nonadded %O values.

or other differences in feeding among predators, and the use of the most common measures of dietary overlap to detect differences between diets; (*iv*) comment on the reporting of stomach content data and discuss the influence of sampling gear type, experimental design, and statistical analysis when studying diel feeding chronology; (*v*) review the main approaches for estimating daily ration in fishes, identify those most appropriate for sharks, and emphasize the need for including error analyses of the estimates; and (*vi*) discuss the appropriateness of the statistical criteria used to evaluate the fit of mathematical curves in studies of gastric evacuation in fishes.

Owing to the diversity of topics covered, separate sections addressing each of the objectives listed above will be presented. Although many of the recommendations and conclusions are applicable to fishes and other marine vertebrates, examples will be drawn using real data sets of stomach contents of sharks and special attention paid to this group of aquatic predators whose study presents unique difficulties.

Quantitative diet analysis

Traditional methods of dietary analysis include counts, frequency of occurrence, and volume or weight of individual prey items (Hynes 1950; Hyslop 1980). Each of these measures provides different insight into the feeding habits of a predator. Numerical abundance is informative regarding feeding behavior (Macdonald and Green 1983), volume or weight measures reflect dietary nutritional value (Macdonald and Green 1983), and occurrence represents population-wide food habits (Cailliet 1977).

A number of compound indices incorporating one or more of these measurements also have been developed. Although the decision of which method to use for determining the relative importance and dominance of the prey is generally subjective (Hyslop 1980), one of the more widely used compound indices in fish diet studies is the index of relative importance (IRI; Pinkas et al. 1971). In this method, the percent frequency of occurrence of each prey category is multiplied by the sum of the percentage volume (or weight) and percentage number. The rationale for using this method lies in that it seems to cancel out biases in its individual components (Bigg and Perez 1985). By incorporating bulk, amount, and occurrence into a single measure it appears to provide a more accurate description of dietary importance and is also intended to facilitate comparative studies. Macdonald and Green (1983), however, argued that compound indices may be redundant, adding little new information to single measures.

Generally, results of dietary analyses are given in tabular form and include one or more of the above-mentioned indices: percentage by number (%N), percentage by volume (%V) or by weight (%W), percentage frequency of occurrence (%O), and IRI. Some studies fail to report %O and IRI for the higher taxonomic levels, complicating or impairing diet comparisons among species. This lack of reporting arises because %O (the proportion of stomachs containing a specific prey category) is a nonadditive index (several different categories of a specific prey type, e.g., several families of teleosts, can occur simultaneously in the same stomach, be counted more than once, and yield a total for the taxonomically higher prey type (all teleosts) >100%). Furthermore, if these values of %O are used in the calculation of IRI, the IRI value itself is also affected. For example, the sum of %O values corresponding to each of 14 families or categories of fishes in stomachs of young lemon sharks (*Negaprion brevirostris*) (Cortés and Gruber 1990) is 131.2%, equivalent to an absolute IRI value of 22 802 (Table 1). However, recalculation of %O and IRI for teleosts as a higher taxonomic group yields values of 86% and 14 947 for %O and IRI, respectively (Table 1).

Because they are not expressed as a percentage, IRI values make comparisons difficult among food types. Although IRI has been expressed occasionally on a percent basis (e.g., Carrassón et al. 1992; Barry et al. 1996; Cortés et al. 1996), this is not yet standard practice. It is therefore suggested that IRI be expressed on a percent basis, such that %IRI for a specific food category i (IRI_i) becomes

$$\%IRI_i = 100\, IRI_i / \sum_{i=1}^{n} IRI_i$$

where n is the total number of food categories considered at a given taxonomic level. Percent IRI values calculated with added %O values (the sum of %O values for all prey categories at a given taxonomic or grouping level) and nonadded %O values (%O value for the higher taxonomic or grouping level) in the above example yield 97.2 and 96.6%, respectively. Similarly, %IRI values calculated with both methods

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for crustaceans in the diet of the bonnethead shark (*Sphyrna tiburo*) yield 82.2 and 84.9%, respectively, whereas absolute IRI values calculated with both methods yield 14 250 and 13 006, respectively (Table 1). On the basis of this limited analysis of the relative performance of IRI and %IRI, the latter seems more robust to changes in %O values and could be used as a standardized index to facilitate diet comparisons.

Thus, to promote consistency and facilitate comparison among studies, and to obtain a robust estimate of relative importance of the prey, whenever possible results of dietary analyses should be reported as %N, %W (or %V), %O, and %IRI for all taxonomic levels considered.

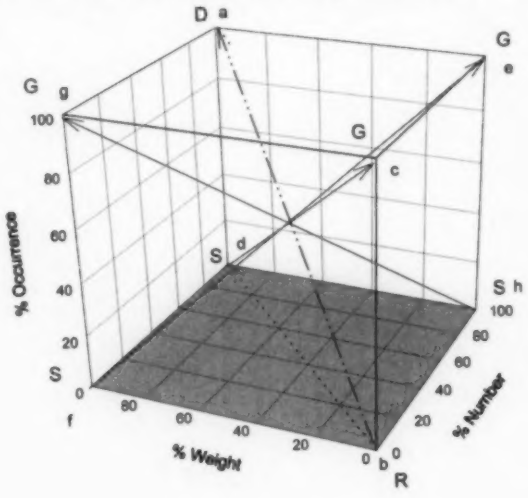
A new graphical representation of the diet

Graphical representation of diet analysis has been used as an alternative to summary tables, because results are easier to interpret (Cailliet et al. 1986; Mohan and Sankaran 1988; Cailliet and Ebeling 1990; Costello 1990). Costello (1990) presented a graphical method that uses the frequency of occurrence and a percent measure of abundance (%W, %V, or %N) and provides a good depiction of prey importance (dominant or rare), predator feeding strategy (specialized or generalized), and the degree of homogeneity of feeding in the predator population. Tokeshi (1991) proposed the use of diversity indices to illustrate feeding strategies also at the population level. In contrast, Bridcut and Giller (1995) proposed an individual-based method that utilizes individual prey type diversity and consumption levels to characterize feeding strategies.

I propose a modification of Costello's method, which uses %O, %N, and %W (or %V) in a three-dimensional graphical representation of population-level stomach content data. Each point on the graph represents the percent occurrence and abundance (in weight or volume and numbers) for a prey category (Fig. 1). Any prey point located close to 100% O, 100% W, and 100% N is the dominant food taxon or category (*a* in Fig. 1). Conversely, points located near the origin of the three axes represent rare prey types (*b* in Fig. 1). Any point located closer to the %N axis than to the %W axis along the horizontal plane indicates that counts contribute more than weight to the abundance of that item. Conversely, any point located closer to the %W axis than to the %N axis along the horizontal plane indicates that weight contributes more than counts to the abundance of that item.

The other six vertices of the cube in Fig. 1 can be regarded as extreme cases pointing to either specialized or generalized diets. Thus, a cluster of points located close to 100% O and the origin of the other two axes (%W and %N) would indicate a generalized diet (most predators take several different prey taxa in low abundance; *c* in Fig. 1). In contrast, a point close to 1% O, 100% W, and 100% N (*d* in Fig. 1) would indicate a specialized diet by a few predators, which would take large numbers of heavy items or items that make up a very large proportion of the total number and weight of stomach contents. A point located close to 100% O, 100% N, and 1% W would be indicative of a light food item consumed by most predators (*e* in Fig. 1). Conversely, a point close to 1% O, 1% N, and 100% W would indicate a specialized diet by a few predators, which would take a few very heavy items or items that make up a very large proportion of the total weight of stomach contents (*f* in Fig. 1). A point located near 100% O, 100% W, and 1% N (*g* in Fig. 1) would indicate that most predators take a few heavy items or items that make up a very large proportion of the total weight of stomach contents. In contrast, a point close to 1% O, 1% W, and 100% N (*h* in Fig. 1) would indicate a specialized diet by a few predators, which would take very large numbers of light items.

Fig. 1. Proposed three-dimensional graphical representation of stomach content data. D, dominant food category; R, rare food category; G, generalized diet; S, specialized diet. See text for definitions of *a*, *b*, *c*, *d*, *e*, *f*, *g*, and *h* and explanation of lines.



As proposed by Costello (1990) for his two-dimensional graphical analysis, diagonals can also be drawn on the three-dimensional plot for the present method to help visualize prey importance (dominant versus rare prey taxa) and predator feeding strategy (generalized versus specialized feeding). Thus, a line uniting points *b* and *a* in Fig. 1 would indicate increasing prey importance, and lines uniting points *d* and *c*, *f* and *e*, and *h* and *g* would all indicate a shift from a specialized to a more generalized feeding strategy.

Illustrative examples

The graphical framework presented in Fig. 1 can be illustrated with several examples of shark diets. The diet of the lemon shark (Cortés and Gruber 1990; Fig. 2*a*) is dominated by teleosts, while crustaceans, molluscs, and plants are rare. Weight contributes more to the overall abundance of teleosts (point situated to the left of the floor diagonal), while counts contribute more to the overall abundance of plants. Feeding in the lemon shark is homogeneous, with most predators specializing in teleost prey. In contrast, the diet of the blue shark (*Prionace glauca*) (Harvey 1989; Fig. 2*b*) is much more heterogeneous. Teleosts are consumed by about half of the predators in small numbers but constitute half the total weight of stomach contents. Some blue sharks specialize on crustaceans, which they consume in very large numbers, while most of the blue sharks eat a few molluscs (cephalopods). Elasmobranchs and plants are rare items. Overall, the diet of blue sharks is generalized.

The proposed graphical method can also be used to show ontogenetic, seasonal, geographic, or habitat-related dietary

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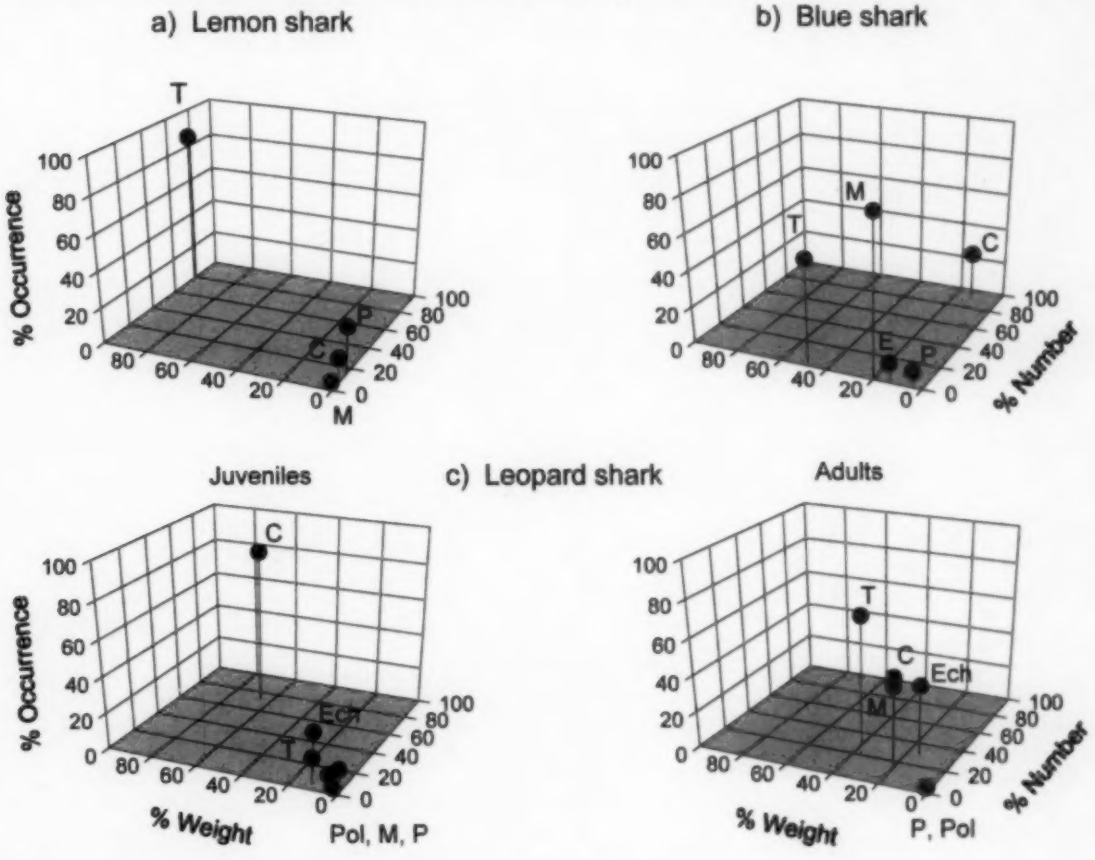
Fig. 2. Four examples of shark diets used to illustrate the three-dimensional graphical representation of stomach content data: (*a*) lemon shark (*Negaprion brevirostris*), (*b*) blue shark (*Prionace glauca*), and (*c*) juvenile and adult leopard shark (*Triakis semifasciata*). T, teleosts; P, plants; C, crustaceans; M, molluscs; E, elasmobranchs; Ech, echinoderms; Pol, polychaete worms.



differences in a predator population or species. For example, the leopard shark (*Triakis semifasciata*) (Talent 1976; Figs. 2*c* and 2*d*) exhibits an ontogenetic dietary shift. The dominant prey item in the diet of juvenile leopard sharks is crustaceans, while echinoderms, teleosts, polychaete worms, molluscs, and plants are only rarely found (Fig. 2*c*). Adult leopard sharks have a more generalized diet (Fig. 2*d*). Teleosts and molluscs become more important, and crustaceans less important, in the diet of the adult sharks. This graphical representation of the diet allows us to easily identify the dominant and rare prey items, generalized versus specialized feeding, and homogeneous versus heterogeneous diets.

Statistical description of feeding habits

Precision in diet description, sample size sufficiency, and dietary comparisons

Ferry and Cailliet (1996) recently reported that, from the over 200 dietary studies they reviewed, none provided any estimates of precision in describing diet. These and other authors (Huturbia 1973; Cailliet 1977; Hoffman 1979; Karpov and Cailliet 1979; Barry et al. 1996, among others) have advocated the use of cumulative prey curves or trophic diversity curves to determine whether a sufficient number of samples has been collected to precisely describe the diet of a particular predator. Essentially, in these approaches the cumulative number of (preferably) randomly pooled stomachs (*X* axis) is plotted against the cumulative number of prey types or a cumulative measure of trophic diversity (*Y* axis), with the asymptotic stabilization of the curve indicating the minimum number of stomachs that have to be analyzed to obtain precise and thus more reliable results.

For subsequent dietary comparisons between species or other factors, Ferry and Cailliet (1996) strongly advocated the use of a priori power analysis (Cohen 1988) to determine sample size sufficiency, a technique that apparently has not been used in fish food habit studies.

Many studies of fish diets attempt to describe variations in stomach contents owing to season, age, size, location, habitat, or other factors but often include only qualitative comparisons, with no statistical support for their conclusions. Crow (1979) first proposed the use of multivariate statistical methods, in which prey categories are treated as separate variates, to test for dietary differences when stomach contents are expressed as gravimetric proportions. Somerton (1991) further proposed

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a nonparametric randomized test for a one-way multivariate analysis of variance (MANOVA) to test for differences in diets between two samples also expressed in terms of weight. In this latter approach, significance was tested with Hotelling's T^2 and post-hoc univariate t tests run to detect differences owing to individual prey categories. Similarly, 1-, 2-,. . ., n-way MANOVA can be applied to stomach content weights or volumes (not proportions) with the weight or volume of each food category as a dependent variable and seasons, sizes, locations, or other factors as treatments. For example, in a one-way MANOVA testing for differences in diet composition among seasons, if the F statistic in the multivariate test is found to be significant, then univariate F tests can reveal the dependent variable(s) (weight of food categories) responsible for the significance. Alternatively, canonical discriminant analysis (Manly 1994) can further detect the individual food categories responsible for the variation in each particular season.

When stomach contents are expressed numerically or as occurrences, an analogous approach is to use multiway contingency table analysis based on log–linear models. The significance of the interaction terms (e.g., food type × season) can be tested by individually deleting them from the model and a post-hoc test then can be run to detect specific differences. One constraint of multiway log–linear analysis that limits its applicability is that very large sample sizes are required to have less than 20% of the cells with values <5. Crow (1982) advocated the use of simpler $R \times C$ contingency table analysis to test for independence between food categories and predators or predator characteristics (such as seasons or other factors) when stomach contents are expressed numerically or as occurrences. The advantage of this technique is that it allows one to readily identify the rows (prey types) and columns (e.g., predators) most responsible for the dietary differences. Despite its simplicity this technique is seldom used in fish food studies.

Illustrative example

The results of a three-way contingency table (log–linear) analysis testing for seasonal variations in the diet of four size-classes of bonnethead sharks reveal that all two-factor interactions are significant (Table 2). The significance of each interaction term is determined by testing the significance of the χ^2 value obtained by subtracting the likelihood ratio χ^2 statistic of the original model from the χ^2 of the model without the interaction.

A 4 × 4 contingency table (modified from Cortés et al. 1996) that includes both the χ^2 and G statistics (Sokal and Rohlf 1981; Table 3) is then applied to examine the interaction food type × season. Cell values in this case are numbers of each prey type, but could also be occurrences. To avoid too many cells having expected frequencies <5 (Sokal and Rohlf 1981; Crow 1982; and references therein), some of the prey types were pooled into a larger category (miscellaneous). However, as pointed out by Crow (1982), some expected frequencies <5 should not affect the analysis. The χ^2 and G values were calculated for each row and column. The grand total χ^2 and G statistics indicated a significant difference ($p < 0.001$, df = 9) in the proportions of prey types being consumed seasonally. The rows and columns contributing most to the variability, i.e., those with the largest χ^2 and G marginal values, can readily be identified by examining Table 3. Among seasons (columns), the main source of variation comes from winter; among prey types, the main source of variation comes from digested food, followed by plants to a lesser extent. Expected values (in parentheses) are also included in Table 3 to allow one to readily identify those cells contributing most to the variation.

Table 2. Three-way contingency table (log–linear) analysis testing for seasonal differences in diet composition among four size-classes of bonnethead sharks.

Factor deleted	Likelihood ratio χ^2 statistic	df	Test of interaction	p
None	27.4	27	na	0.444
Food type × season	76.4	36	49.0	<0.001
Food type × size	165.5	36	138.1	<0.001
Season × size	97.8	36	70.4	<0.001

Note: Each two-factor interaction is examined and tested for significance by deleting it from the model. Factor 1, food type (four levels); factor 2, season (four levels); factor 3, size-class (four levels). na, not applicable.

Post-hoc tests (Table 4) reveal that eliminating the main source of variability among rows (digested food) or among columns (winter) does not suffice to yield a nonsignificant χ^2 or G statistic. Nonsignificance is achieved, however, after further eliminating the second largest source of variation among rows (plants) in both cases. Reexamining Table 3, we would conclude that the seasonal dietary differences were mainly the result of a larger than expected amount of digested food in winter and a smaller than expected amount of plants in fall.

Dietary overlap

The degree of overlap in the diet of fish species has often been measured using parametric or non parametric correlation coefficients or other measures of dietary overlap, such as Morisita's measure (Morisita 1959; C), the simplified Morisita index (Horn 1966; C_H), percentage overlap (Renkonen 1938; P_{jk}), and Horn's index of overlap (Horn 1966; R_o).

Cailliet and Barry (1979) and Krebs (1989) discussed the advantages and disadvantages of the most widely used indices of overlap and similarity and Krebs (1989) recommended using Morisita's index as the best measure to minimize bias, and Horn's index if prey types cannot be expressed as numbers. Numerous studies still use rank correlation to compare fish diets. However, the choice of how many food categories to use in the comparisons is left to the investigator. Both the number of food categories considered and the degree of decimal precision can influence the analysis, since rank correlation is a semiquantitative measure that assigns ranks to food categories. Thus, when using rank correlation, different groupings of food categories should be attempted to assess the effects of those grouping choices on the results. In contrast, R_o and C_H, two of the most commonly used indices when resources are expressed as proportions (Cailliet and Barry 1979), are quantitative measures not influenced by the number of food categories considered because they use the relative proportions of each food type rather than assigning ranks. R_o and C_H vary between 0 (no categories in common) and 1 (identical categories). For C_H, Zaret and Rand (1971) considered values >0.60 to indicate significant overlap; no formal statistical testing of C_H or R_o has been developed (Cailliet and Barry 1979).

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Table 3. Contingency table analysis of the seasonal variation of four different categories of food items found in the stomachs of bonnethead sharks.

Prey type	Winter	Spring	Summer	Fall	N_i	χ_i^2	G_i
Crabs	13 (24)	348 (322)	191 (213)	50 (44)	602	10.05	10.99
Miscellaneous	1 (2)	25 (23)	11 (16)	7 (3)	44	6.27	5.32
Plants	13 (11)	128 (148)	124 (98)	11 (20)	276	14.24	14.58
Digested food	12 (3)	30 (38)	25 (25)	4 (5)	71	32.32	18.68
N_j	39	531	351	72	993		
χ_j^2	35.92	6.47	10.72	9.78		62.95***	
G_j	23.10	6.67	10.39	9.40			49.57***

Note: Values are observed numbers, with expected values given in parentheses. The χ^2 and G statistics are highly significant (***, $p < 0.001$).

Table 4. Results of post-hoc contingency table analyses of the seasonal variation of four different categories of food items found in the stomachs of bonnethead sharks after eliminating selected rows and (or) columns.

Row(s) or column(s) eliminated	Significance of χ^2 or G statistic	Sample size
Digested food	$p < 0.001$	922
Digested food, plants	ns	646
Winter	$p < 0.001$	954
Winter, plants	ns	691

Note: ns, not significant.

Table 5. Diet overlap in male and female bonnethead sharks using the Spearman rank correlation coefficient (r_s), the simplified Morisita index (C_H), and Horn's index of overlap (R_o) for different groupings of food categories and varying decimal precision (in parentheses).

No. of food categories	r_s	Critical value	C_H	R_o
7	0.929	0.929[a]	0.992	0.978
17	0.922 (1)	0.711[b]	0.995 (1)	0.947 (1)
17	0.812 (2)	0.711[b]	0.995 (2)	0.946 (2)
17	0.722 (3)	0.711[b]	0.995 (3)	0.946 (3)

[a]Critical value at 1% significance.
[b]Critical value at 0.2% significance.

Illustrative example

Cortés et al. (1996) used the Spearman rank correlation coefficient (r_s) corrected for ties (Fritz 1974), C_H, and R_o to calculate overlap in the diet of male and female bonnethead sharks. If the stomach content data are grouped into seven categories, the null hypothesis (H_o) of no correlation ($\rho = 0$) is rejected at the 0.01 level of significance (Table 5). If, instead, 17 categories are used in the calculation of r_s, H_o is rejected at the 0.002 level of significance and the fewer decimal points considered, the larger our confidence in rejecting H_o, i.e., the more similar the diet becomes. In contrast, results obtained with the quantitative indices C_H and R_o are very homogeneous, relatively unaffected by the number of food categories considered and by decimal precision (Table 5). All C_H and R_o values indicate a very high degree of similarity in the diet.

Stomach content data transformations

Stomach content weight distributions frequently do not satisfy the assumptions of parametric tests (i.e., independence, normality, homogeneity of variances, and additivity). In that case, the original data should be transformed to attempt to satisfy those assumptions. Several transformations have been applied to stomach content weight data: the logarithmic (natural or base 10) transform, the arcsine transform, and the square-root transform (Sokal and Rohlf 1981). Because stomach content weights are generally reported as a percentage of fish body weight (%BW), the arcsine transform, indicated for percentages and proportions (Sokal and Rohlf 1981; Krebs 1989), seems appropriate. Data should be reported as back-transformed means in those cases where transformations were applied. Alternatively, if the parametric statistical assumptions are still not met after the data transformation, especially in those cases where the assumptions are grossly violated, nonparametric techniques should be used and median values reported.

Table 6. Evaluation of relative performance of several transforms for normalizing stomach content weight data of lemon sharks, expressed as percentage of wet body weight (%BW).

Transform	Normality test results	p	Kolmogorov–Smirnov statistic (D)	Mean	Back-transformed mean
None (Y)	Failed	<0.001	0.305	0.515	—
$\ln(Y+1)$	Failed	<0.001	0.249	—	0.350
$Y^{1/2}$	Failed	<0.001	0.185	—	0.231
$(Y+1)^{1/2}$	Failed	<0.001	0.269	—	0.418
$\arcsin(Y/100)^{1/2}$	Failed	<0.001	0.185	—	0.232

Illustrative example

The distribution of stomach content weights (expressed as percentage of wet body weight) in a sample of 86 lemon sharks was found not to be normal (Table 6). Visual inspection of the data plotted graphically as a histogram and probit analysis (Sokal and Rohlf 1981) further revealed substantial deviations from normality. Some of the more common transformations were then used to attempt to normalize the original data and a Kolmogorov–Smirnov test of goodness of fit (Sokal and Rohlf 1981) was run on each transformed data set, yet none of the data transforms yielded normality ($p < 0.001$ in all cases; Table 6). By noting the magnitude of the Kolmogorov–Smirnov statistic (D) and comparing it with the tabulated 1% critical value (0.111), the least significant values (smaller D values) were obtained with the square-root and arcsine transforms, although data were still far from being normally

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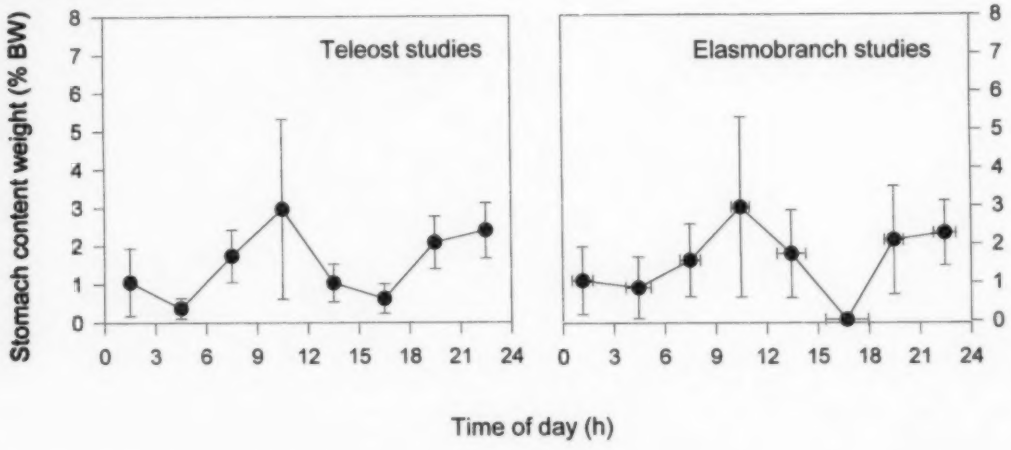
Fig. 3. The influence of experimental design on studies of diel feeding chronology in teleost and elasmobranch fishes. In elasmobranch studies, grouping sampling times of stomachs collected throughout 24-h periods during a sampling campaign into successive 3-h intervals adds variability along the horizontal axis. In teleost studies, stomachs can generally be collected at specific sampling times. Vertical and horizontal error bars are simulated ±SEs.



distributed. Had we found that any of the transformations satisfied the normality assumption, we should still have tested for homoscedacity if we were to compare our sample with other data sets. In this particular example, the median should probably be calculated and nonparametric techniques applied for comparisons with other data sets.

Diel feeding chronology: influence of sampling gear, experimental design, and statistical analysis

Discontinuity of feeding activity over time of day is investigated in fishes by randomly collecting samples at regular intervals throughout the 24-h period (Jenkins and Green 1977). To account for differences in fish size, stomach content weights are usually expressed as a percentage of the fish weight (%BW). Mean %BW values for each sampling time are then calculated and plotted against sampling time in the X axis, with ±SE, 95% confidence intervals (CI), or range generally placed around the mean at each sampling time (Fig. 3). Jenkins and Green (1977) showed that visual inspection of these plots was misleading because apparent peaks in stomach fullness detected by eye are often not corroborated by statistical analyses. These authors proposed one-way analysis of covariance (ANCOVA) of fish weight versus stomach content weight to test statistically for temporal feeding continuity, indicating that the test of intercepts given common slopes is the test of among-time differences in stomach content weights adjusted for fish size.

Diel feeding continuity is more often examined using one-way analysis of variance (ANOVA). This test allows detection of discontinuity in stomach content weight among the sampling intervals when applied to original or transformed stomach content weight data. To further determine which sampling times are responsible for the difference(s), post-hoc Student–Newman–Keuls or similar tests can be applied to perform pairwise comparisons of means. Alternatively, a nonparametric one-way ANOVA (Kruskal–Wallis test) and corresponding post-hoc nonparametric comparisons (Dunn's or similar tests) can be applied. Cortés et al. (1996) compared ANCOVA and ANOVA tests to determine feeding (dis)continuity in bonnethead sharks, finding that the results from the two approaches agreed in <50% of the cases (Table 7).

Sampling gear and experimental design can also affect results for diel feeding chronology and food consumption estimates. Studies dealing with teleost fishes generally use electrofishing, beach seines, traps, otter trawls, or similar gear to sample at regular intervals throughout the day. This type of gear allows the investigator to collect most of the samples for each interval in a short time period and thus obtain a good representation of stomach content weights at each sampling time. In contrast, elasmobranchs are much less abundant than teleost fishes and more difficult to collect. Passive gear, such as gill nets, is generally set throughout the day and retrieved at regular intervals. The exact time of capture of each sampled specimen is generally unknown. In some cases, owing to the difficulty of collecting a substantial sample of stomach contents in a 24-h period, instead of collecting samples at regular intervals (typically 3 h) throughout the 24-h period, sampling times of stomachs collected at different times throughout the day and night during a sampling campaign are grouped into successive (generally 3 h) intervals (Medved et al. 1988; Cortés and Gruber 1990; Cortés et al. 1996), adding variability along the horizontal axis (Fig. 3). Cortés et al. (1996) showed that in this type of experimental design the length (3 versus 4 h) and starting time of the interval yielded different results on diel feeding chronology within and between statistical tests (Table 7).

The effect of active versus passive gear in studying diel feeding chronology and estimating food consumption has not been investigated in elasmobranchs. Hayward et al. (1989) found significantly higher median amounts of food in stomachs of yellow perch (*Perca flavescens*) caught with passive gear (gill net) versus active gear (otter trawl). They concluded that active gear produced more accurate estimates of food consumption because it samples both low-activity or nonforaging

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Table 7. Summarized results from a study by Cortés et al. (1996) showing the influence of the statistical test and the length and starting point of the time interval on the determination of diel feeding chronology.

Time interval length (h)	Time interval starting point	Statistical test results (p)	
		ANOVA	ANCOVA
3	11:00	<0.05	ns
3	12:00	<0.05	ns
3	13:00	ns	ns
4	10:00	<0.05	ns
4	11:00	<0.05	<0.05
4	12:00	<0.05	<0.01
4	13:00	ns	ns

Note: ns, not significant.

fish and actively feeding fish, whereas passive gear only effectively samples those individuals in a population that are actively feeding. Active gear, such as otter and bottom trawls, has been used in dietary studies of skates, rays, and bottom-dwelling sharks but is not appropriate for sampling most coastal and oceanic shark species. To sample these species, gill nets are the most commonly used type of gear, largely preferable over longlines because the latter tend to attract individuals with empty stomachs (Wetherbee et al. 1990). Passive versus active gear bias in field estimation of diel feeding chronology and food consumption could probably be investigated in some species of skates and rays but this does not appear to be feasible for most species of sharks.

An important consideration when interpreting results obtained through the standard methods based on sampling at regular intervals throughout the 24-h period is that the terms continuity and discontinuity are often misapplied or misunderstood. Considering only the weight of stomach contents does not allow one to draw any conclusions on feeding (dis)continuity; only conclusions on feeding intensity and chronology can be drawn at best. For example, when an investigator concludes that feeding is continuous after analyzing stomach content weight versus time of collection data, all that can actually be concluded is that there were no significant differences in the weight of food in the stomach over a diel period. However, feeding may still be discontinuous or, rather, intermittent, in the species under study. Similarly, if, for example, the mean weight of stomach contents of a given fish species is found to be significantly higher at 10:30 (Fig. 3), this does not necessarily mean that feeding activity increases at that time in that species, unless we can determine that all food items were consumed very recently.

Stomach content weights alone do not suffice to assess feeding continuity or to interpret diel feeding chronology. We also need to consider the percentage of empty stomachs in the sample and the dietary composition and stage of digestion of each individual item in each individual stomach at each sampling interval. If the digestion rate of each different prey item is known or can be approximated, meal sizes and ingestion times can be reconstructed. The distribution of ingestion times among time intervals then can be tested with a goodness of fit test to detect preferred feeding times in the population studied. These considerations are especially important in large carnivorous predators such as sharks, most of which only feed intermittently and not necessarily every day (Wetherbee et al. 1990). Diel feeding activity is a complex process influenced by environmental, behavioral, and physiological constraints. Thus, in addition to considering sampled stomach content weights, a better understanding of all these integrated factors is required.

Table 8. Main requirements of the most common in situ methods used to estimate daily ration in fishes.

Model	Gastric evacuation requirements	Periodic sampling	Applicability to elasmobranchs
Elliott–Persson	Exponential	Yes	Yes, if TI ≤ 3 h
Eggers	Exponential	No	Yes
Diana	Any	No	Yes
Olson–Mullen	Any	No	Yes
Pennington	Nonlinear	Yes	Yes

Note: TI, time interval.

Daily ration

The adequacy of the most common models used to estimate daily ration and of the associated types of gastric evacuation curves in fishes has been discussed at length (Elliott and Persson 1978; Mullen 1986; Persson 1986; Boisclair and Leggett 1988; Ruggerone 1989; Boisclair and Marchand 1993; Bromley 1994; and references therein). However, the extent to which the most common models used to estimate daily ration in teleost fishes are applicable to elasmobranchs has not been investigated in depth. Surprisingly, only two published studies to date have used multiple in situ methods to estimate daily ration in elasmobranchs (Medved et al. 1988; Cortés and Gruber 1990). Medved et al. (1988) used the Elliott and Persson (1978) and the Diana (1979) models to calculate the daily ration of the sandbar shark (*Carcharhinus plumbeus*), whereas Cortés and Gruber (1990), in addition, used the Olson and Mullen (1986) model to estimate the daily ration of the lemon shark. Results from these two studies indicated that despite the nonfulfillment of several of the model assumptions, the models used in each study yielded similar estimates of daily ration.

Elliott and Persson (1978) originally proposed two methods for nonpiscivorous fishes, one that assumes constant feeding and another that assumes a decreasing feeding rate with time. Both methods assume an exponential gastric evacuation rate. The second method, in addition, requires an estimate of satiation ration and that the larger fish feed to satiation, making it inadequate or difficult to apply in most situations. The Elliott–Persson method generally referred to in the literature is thus the simpler method that assumes constant feeding rate. Elliott and Persson (1978) empirically determined using the brown trout (*Salmo trutta*) that this method is applicable to intermittent feeders if the sampling interval is kept short (≤3 h). Indeed, despite the violation of the major assumptions of continuous and constant rate of feeding implicit in this method, it seems to yield reasonable results when applied to elasmobranch studies if sampling intervals are kept ≤3 h (Medved et al. 1988; Cortés and Gruber 1990; Table 8).

Another commonly used model to estimate daily ration is that of Eggers (1979). This method also requires an exponential evacuation rate, but only an estimate of the mean weight

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Table 9. Point, jackknife, and bootstrap estimates of daily ration for lemon sharks through the Eggers and the Elliott–Persson models using transformed and original (percent wet body weight) data.

Estimate	Recombinations	Eggers			Elliott–Persson		
		Arithmetic mean	Geometric mean	BT arcsine mean	Arithmetic mean	Geometric mean	BT arcsine mean
Point		2.16	1.47	1.01	2.18	1.56	3.53
Jackknife		2.21 (1.29–3.13)	1.46 (0.95–1.97)	0.44 (0–3.95)	2.23 (1.18–3.28)	1.48 (0.81–2.15)	3.56 (3.08–4.04)
Bootstrap	100	2.27 (2.17–2.37)	1.51 (1.46–1.56)	1.85 (1.52–2.17)	2.25 (2.16–2.33)	1.66 (1.60–1.71)	3.99 (3.83–4.14)
	200	2.20 (2.13–2.27)	1.79 (1.75–1.84)	1.78 (1.55–2.01)	2.29 (2.23–2.35)	1.69 (1.65–1.73)	3.97 (3.86–4.09)
	300	2.17 (2.11–2.23)	1.89 (1.85–1.92)	1.69 (1.50–1.87)	2.26 (2.21–2.31)	1.70 (1.66–1.73)	4.02 (3.92–4.11)
	400	2.15 (2.10–2.20)	1.93 (1.91–1.96)	1.67 (1.51–1.83)	2.23 (2.19–2.27)	1.73 (1.70–1.76)	4.15 (4.07–4.23)
	500	2.14 (2.10–2.19)	1.96 (1.94–1.99)	1.65 (1.51–1.79)	2.22 (2.19–2.26)	1.72 (1.69–1.74)	4.12 (4.05–4.20)
	600	2.14 (2.11–2.18)	1.98 (1.96–2.00)*	1.63 (1.50–1.75)	2.23 (2.20–2.27)	1.71 (1.69–1.73)	4.10 (4.04–4.17)
	700	2.14 (2.11–2.18)	2.00 (1.98–2.02)*	1.66 (1.54–1.78)	2.23 (2.20–2.27)	1.70 (1.68–1.72)	4.09 (4.03–4.15)
	800	2.14 (2.11–2.17)	2.01 (1.99–2.03)*	1.64 (1.53–1.75)	2.24 (2.21–2.27)	1.70 (1.68–1.72)	4.07 (4.02–4.13)
	900	2.14 (2.11–2.17)	2.02 (2.00–2.03)*	1.61 (1.51–1.72)	2.25 (2.22–2.28)	1.70 (1.68–1.72)	4.04 (3.98–4.09)
	1000	2.14 (2.11–2.16)	2.02 (2.01–2.04)**	1.61 (1.51–1.71)	2.25 (2.22–2.27)	1.69 (1.67–1.71)	4.03 (3.98–4.08)

Note: Values in parentheses are 95% confidence intervals. Asterisks indicate significant differences from the point estimate (*, $p < 0.05$; **, $p < 0.01$). BT arcsine mean is the back-transformed mean of data transformed using arcsin $(\%BW/100)^{1/2}$. See text for details.

of stomach contents over a 24-h period (Table 8). Although not calculated by Cortés and Gruber (1990), daily ration by the Eggers method for the lemon shark yields almost identical results to the Elliott–Persson method (2.16 versus 2.18% BW).

The reconstructive method of Diana (1979) seems more appropriate for top predators such as sharks because it assumes that feeding is asynchronous and intermittent, feeding bouts are short, and the interval between meals is longer than digestion time. In addition, gastric evacuation can be of any form (Table 8). The Olson and Mullen (1986) model was also conceived for predatory fish, such as tuna, and assumes that fish feed on mixed prey items at random intervals. Like the Diana model, the Olson–Mullen model is less restrictive than the two exponential models, because any type of gastric evacuation function is acceptable (Table 8). Finally, the Pennington (1985) model assumes that the rate of evacuation at any given time depends only on the stomach contents at that moment, and not on initial meal size. For exponential evacuation rates, the Pennington model simplifies into the Eggers model. The only restriction of this method is that it does not allow for a linear rate of gastric evacuation (Table 8).

The Elliott–Persson and Pennington models allow estimation of the diurnal feeding cycle (Mullen 1986) but are logistically more demanding than the other methods because they require periodic sampling (Table 8). On the basis of fulfillment of assumptions and sampling requirements, it appears that the less restrictive Diana and Olson–Mullen methods are more appropriate for top predators such as sharks.

Analysis of statistical variability in daily ration estimates is usually absent from fish food consumption studies because the models traditionally used to estimate daily ration are deterministic. As an alternative approach, numerous studies have attempted to determine daily ration through different methods and then compare the various estimates derived. Worobec (1984) and Pennington (1985) first proposed equations to estimate an approximate standard error of the daily ration when using the Elliott–Persson and Eggers models, respectively. As discussed by Boisclair and Leggett (1988), the formulae presented by these two authors are restricted by their complexity, combination of transformed and nontransformed variables, and lack of replicated estimations of daily ration. More recently, several studies have used resampling techniques, such as the jackknife or the bootstrap, or Monte Carlo simulations, to provide a measure of the error associated with daily ration estimates (Boisclair and Leggett 1988; Hayward 1991; Hayward et al. 1991; Boisclair and Marchand 1993).

An illustrative example of the measurement of variation in daily ration estimates of elasmobranchs

No error analysis dealing with the daily ration of elasmobranchs has been published, and thus no statistical testing of differences between daily ration estimates has been possible. To provide a first measure of the uncertainty associated with estimating daily ration in sharks, I used the jackknife and bootstrap methods on transformed and original (standardized as percent wet body weight) stomach content data of lemon sharks and compared the estimates obtained through the Eggers and Elliott–Persson models (Table 9).

For the Eggers model, the bootstrap arithmetic mean, geometric mean, and back-transformed mean of arcsin $(\%BW/100)^{1/2}$ (back-transformed arcsine mean) converged on a value of 2.14, 2.02, and 1.61% BW after 500, 900, and 900 recombinations, respectively. Precision (measured as the magnitude of the CI) was higher for the bootstrap estimates than for the jackknife estimate in all cases and increased with number of recombinations. Significant differences with the point estimate were only found for the bootstrap estimates after 600 recombinations when using geometric means (Table 9).

For the Elliott–Persson model, only the bootstrap arithmetic mean converged on a stable value, after 900 iterations (Table 9). Precision was higher for the bootstrap estimates than for the jackknife estimate in all cases, increasing with number of recombinations. No significant differences were found between the jackknife or bootstrap estimates and the point estimate when using any of the three means.

Point, jackknife, and bootstrap (for 1000 recombinations) estimates were compared between the two models for each of the three types of means. Using the overlap of the 95% CI as the significance criterion, significant differences were found between the bootstrap estimates for arithmetic, geometric, and back-transformed arcsine means. Additionally, for geometric means, the Eggers bootstrap estimate was significantly different

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Table 10. Goodness of fit of several mathematical models fitted to gastric evacuation data ($n = 52$) for the lemon shark.

Model	r^2	F	p	RSS	SD of residuals	Y intercept	CV of residuals	Residual analysis[a] N	H	A[b]
Linear	0.607	79.88	<0.001	7046	11.6	96.6	0.177	P ($p = 0.122$)	F ($p < 0.001$)	2.198
Exponential[c]	0.492 (0.615)[d]	50.38	<0.001	2.93	0.24	103.0	0.059	F ($p = 0.002$)	F ($p < 0.001$)	2.273
Exponential	0.615			6902	11.5	102.2	0.176	P ($p = 0.198$)	F ($p < 0.001$)	2.259
Square root[c]	0.561 (0.613)[d]	66.14	<0.001	33.77	0.81	99.1	0.102	F ($p = 0.048$)	F ($p < 0.001$)	2.243
Square root	0.613			6938	11.5	99.3	0.176	P ($p = 0.176$)	F ($p < 0.001$)	2.239
Logistic	0.670			6643	11.3	54.7	0.172	P ($p = 0.100$)	F ($p < 0.001$)	2.391
Gompertz	0.663			6747	11.4	60.7	0.174	P ($p > 0.2$)	F ($p < 0.001$)	2.338

[a]N, normality test; H, homoscedacity test; A, autocorrelation test; P, pass; F, fail.
[b]Autocorrelation tested by Durbin–Watson statistic (values closer to 2 indicate lower autocorrelation of residuals).
[c]Linearized models.
[d]Values are r^2 between back-transformed fitted values ($\hat{Y}_i$) and untransformed observed values (Y_i).

($p < 0.05$) from the Elliott–Persson point estimate, and, for back-transformed arcsine means, the Eggers point estimate was significantly different from both the Elliott–Persson jackknife ($p < 0.001$) and bootstrap ($p < 0.001$) estimates (Table 9).

In all, there was little variation in daily ration estimates among estimation procedures or between models when using arithmetic means. However, there was more substantial variation when using back-transformed means, especially in the case of back-transformed arcsine means (Table 9). This indicates that there are potentially important differences in the accuracy of daily ration estimates obtained from different models and (or) estimation procedures. The present exercise, however, was not designed to identify which estimates are more accurate.

Resampling techniques provide a picture of the variability associated with estimating daily ration because they allow calculation of CIs and comparison of estimates obtained through different models. For the data set studied, a greater precision was achieved with the bootstrap than with the jackknife method in all cases, and precision always increased with increasing number of recombinations. On the basis of precision alone, the bootstrap method seems preferable over the jackknife to provide a measure of the error associated with estimating daily ration.

Gastric evacuation

Knowledge of gastric evacuation rates in fishes is essential to estimate daily ration through in situ methods. In elasmobranchs, especially in sharks, measurement of evacuation rates under controlled laboratory or field conditions has been severely impaired by the difficulty of collecting and keeping large or adult specimens in captivity. Thus, researchers have opted for a more pragmatic approach and concentrated on small species (Jones and Geen 1977) or juvenile specimens of larger species (Medved 1985; Cortés and Gruber 1992). In all, knowledge of gastric evacuation dynamics in elasmobranchs remains very fragmentary.

The adequacy of the most common models used to describe gastric evacuation patterns in fishes (linear, exponential, square root, and surface area) is still a matter of research and debate (Bromley 1994; Gerking 1994). In general, no single model appears to fit equally well the evacuation dynamics of different species consuming different prey items under varying environmental conditions. While the physiological rationale of the different curves has been reviewed (Jobling 1981, 1986, 1987; Persson 1986; Gerking 1994), the appropriateness of the criteria used to select the best model of evacuation from a statistical standpoint has only been partially discussed (Bromley 1994). Some of the most commonly used criteria include comparison of r or r^2 (Macdonald et al. 1982; Persson 1982, 1984; Brodeur and Pearcy 1987; Bromley 1987; Ruggerone 1989; Dos Santos and Jobling 1991; Russell and Wooton 1993; Bromley 1994), residual sum of squares (RSS) or residual mean squares (RMS; Medved 1985; Jobling 1987), standard deviation (SD) or variance of the residuals (Longmuir et al. 1971; Jobling 1986), and the coefficient of variation of the residuals (CV; Somerton 1980; Cortés and Gruber 1992).

A problem typically arises when exponential and square-root models are linearized to perform least-squares regression, because the magnitudes of the above criteria are no longer comparable. One way to circumvent this problem is to apply nonlinear regression and use the above criteria to compare fits. Preferably, a criterion adjusted for the number of observations and parameters in the model (adjusted linear r^2) and the magnitude of the dependent variable (adjusted nonlinear r^2 (Dos Santos and Jobling 1991) or CV (Somerton 1980)) should be used. An alternative is to calculate an adjusted r^2 between the back-transformed fitted values ($\hat{Y}_i$) and the untransformed observed values (Y_i) for the linearized model, since it yields the same result as the nonlinear r^2 value calculated for the corresponding nonlinear model.

Additional criteria that should be used to compare model fits include determination of the Y-axis intercept (Persson 1982; Jobling 1986, 1987; Brodeur and Pearcy 1987; Ruggerone 1989; Cortés and Gruber 1992) and an examination of residual plots (Medved 1985; Ruggerone 1989) or formal residual analysis (Olson and Mullen 1986). Compliance of each model to regression assumptions is important not only as a comparative criterion but also to determine whether a weighting factor needs to be applied to the data.

Illustrative example

Gastric evacuation data for the lemon shark were fitted with several common models and their goodness of fit was evaluated with several criteria (Table 10). The fit of the linear model cannot be compared with that of the linearized exponential or square-root models using r^2, RSS, SD of residuals, or CV as criteria because linearization has altered their magnitudes

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(Table 10). All F values from the ANOVA table are highly significant, indicating that the models explain most variance. When comparing the linear with the linearized models, the most accurate estimate of Y-axis intercept is given by the square-root model, but residual analysis reveals that only the linear model passes the normality assumption of least squares regression and has the lowest autocorrelation of residuals. However, if we consider the nonlinear exponential and square-root models, both have slightly better fits than the linear model according to r^2, RSS, SD of residuals, and CV and predict more accurately the Y intercept, while also conforming to the normality assumption of nonlinear least squares regression. The fit of the nonlinear exponential and square-root models has thus changed in relation to that of the linearized forms. Note that the adjusted r^2 value between back-transformed fitted values and observed values of the linearized exponential and square-root models equals the adjusted nonlinear r^2 value of the corresponding nonlinear form (Table 10). While the logistic and Gompertz models show a better fit than all of the other models according to r^2, RSS, SD of residuals, and CV, they severely underestimate the Y intercept and are thus inadequate.

Because none of the models passed the homogeneity of variances assumption of least squares regression and on the basis of the direct relationship between σ_i^2 and X_i observed in the analysis of residuals, a weight $w_i = 1/X_i$ (Neter et al. 1990) was applied to the data and the fits were reexamined. In this particular example, weighting does not improve the fit; it actually worsens it because the normality assumption of regression is not met. Overall, considering the unweighted fits (Table 10), it does not seem practical to select a best-fitting model on the basis of the criteria used, probably as a result of insufficient experimental data.

Inconclusive outcomes following efforts to identify a best-fitting gastric evacuation model may arise quite commonly. If the gastric evacuation model is to be further used in the estimation of daily ration through in situ methods, a prudent approach is to use several in situ methods with different gastric evacuation requirements or methods that accommodate any form of gastric evacuation and then compare the outcomes corresponding to each gastric evacuation form used.

Summary

Published studies of diets, food habits, and food consumption of fish show a lack of standardization in the use of indices and of consistency in methods, experimental design, data reporting, and statistical analysis, all of which hinder comparative studies.

The need for consolidation of methods is especially critical in elasmobranch feeding studies, owing to the added difficulty of obtaining large sample sizes and the limited ability to conduct controlled experiments with large or adult specimens. A pragmatic approach is recommended in food consumption studies of elasmobranchs to predict diel feeding chronology and feeding and gastric evacuation rates. To advance our knowledge in these areas, we may have to compromise between the rigorous application of experimental design and adherence to model assumptions and other approaches that require fewer parameter estimates and are logistically less demanding. Emphasis should then be placed on maximizing the information derived from examining stomach contents of field-caught fish. For example, when attempting to predict daily ration, multiple models should be applied and resampling techniques, such as the bootstrap, should be used to provide a measure of the error associated with parameter estimates. Results from gastric evacuation studies also should be fully incorporated into attempts to describe diel feeding chronology. New food consumption models more akin to the feeding activity of large predators such as sharks may well have to be developed. Setting aside logistical considerations, the main needs in feeding studies of elasmobranch fishes remain a more quantitative approach and the consolidation of methodology and data analysis.

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DISCUSSION

Comment — The prediction of hypolimnetic oxygen profiles: a plea for a deductive approach[1]

Lewis A. Molot

Livingstone and Imboden (1996) presented a generalized deductive model to predict oxygen profiles in stratified lakes. Their model is an excellent contribution to the literature and is deserving of further exploration of form and application. The authors used the presentation of their model to make the argument that research efforts should, in general, be directed towards deductive (theoretical or mathematical) rather than inductive (empirical or statistical) modeling because of the greater potential benefits to the research community and others. While deductive models have advantages as outlined by the authors, I do not agree with the authors' dismissal of empirical models and their contention that deductive models should always be the approach of choice when there is sufficient understanding of the underlying processes. There are a variety of reasons for model building and proposed uses for a model may influence its structure. An example illustrates why different approaches are necessary.

The main impetus for the development of AHOD (areal hypolimnetic oxygen deficit) and oxygen profile models in Canada and elsewhere has been the imminent threat to water quality posed by changes in land use, i.e., development. To be useful as an environmental management tool and to be taken seriously by regulatory agencies an oxygen model must meet two essential criteria (at least) regardless of its structure: it must be accurate because of the financial, legal, and environmental stakes involved, and it must include a measurable value relevant to the problem at hand as an independent (in statistical jargon) or driving (in mathematical jargon) variable. Stated in a different way, a management model should accurately predict the effects of a proposed development on oxygen concentrations as a means of assessing the potential impact of the development. Ideally, permits will be issued if the predicted impact does not exceed published government standards. Phosphorus is typically used in the case of eutrophication because it provides a dynamic link between land use activities and water quality.

The Livingstone and Imboden model in its current form cannot predict the effects of development on oxygen profiles and hence does not meet the second criterion. Presumably their model might be modified by expanding the function J_A, the oxygen consumption rate associated with sediments, to make it an explicit function of phosphorus. The modification might be accomplished by regression or deductive approaches, although ultimately even deductive models must have their constants empirically fit (thus blurring the distinction between inductive and deductive models). However, there is no guarantee that a modified model will meet the first criterion: that it be accurate. If a modified version of their model is tested and deemed insufficiently accurate for management purposes, what then? What resources are left at risk while we wait for an effective deductive management tool that satisfies researchers because of its possible hypothesis-generating abilities? Clearly, Livingstone and Imboden's argument that the deductive "approach may be preferable even if the predictions made by such a deductive model turn out in certain cases to be less accurate statistically than short-cut predictions made by inductive models based on limited data" should be reconsidered. It ignores the realities of environmental management: that managers will not be persuaded to adopt a deductive model in the absence of accuracy, even if its form is preferred by (some) researchers. In this case, function takes precedence over form. Empirical models have often proven effective and their value as management tools should not be minimized because of a preference for a different type of internal model structure. Clearly, the development of deductive models should not preclude efforts to develop effective empirical models when specific and pressing problems demand immediate attention. Moreover, both inductive and deductive models can be readily developed using limited data; the resulting predictions are not "short-cut" as much as they are uncertain.

Over the years, much has been published on the subject of deductive versus inductive or theoretical versus empirical approaches in environmental science. I argue here that the debate between advocates of either approach is somewhat sterile; we should be advocates for both. Like it or not, there is room for both approaches according to need and personal predilection, and science is much richer for it. Ultimately, however, science is competitive and the merits of a particular model will determine its status and fate amongst scientists and other end users. If certain types of models remain in favour, I can only surmise that it is because they have been generally successful.

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Reply — The prediction of hypolimnetic oxygen profiles: a plea for a deductive approach[1]

David M. Livingstone and Dieter M. Imboden

While not agreeing completely with Molot's (1997) comments on our oxygen depletion paper (Livingstone and Imboden 1996), we appreciate having the opportunity to respond to lucid and practical criticism of our plea for a deductive, process-based approach to the prediction of hypolimnetic oxygen profiles. We note with pleasure that an airing of the tacit assumptions and preconceptions underlying various attitudes towards tackling environmental problems is still able to stimulate fruitful and constructive debate.

There are many points of convergence between Molot's opinions and our own, especially our common belief that both inductive and deductive approaches are indispensable to the environmental sciences. Where we differ is principally in the way we believe these approaches and their associated models can be best made use of. In our opinion, the decision on which approach to use should not be based merely on personal preference. Inductive and deductive approaches are qualitatively different (although deductive models can and do incorporate inductive elements and vice versa; see below) and have different aims. Our concern is not that a deductive approach be adopted under all circumstances but rather that the approach chosen be appropriate to the problem tackled. We have no objection to an inductive approach with purely empirical curve fitting when this is justified (see Livingstone and Imboden 1993, for instance), and we would, of course, be just as ready to argue against the employment of deductive models in specific cases.

Molot interprets our opinion as being that "deductive models should always be the approach of choice when there is sufficient understanding of underlying processes," and then criticizes this opinion on the grounds that environmental managers need accurate predictive tools with an external independent or driving variable. We believe that neither deductive nor inductive models designed to deal with environmental problems can afford to sacrifice predictive accuracy by ignoring already available knowledge, either of data or of processes. In deductive models, relevant processes that are badly or incompletely understood are not omitted but parameterized empirically (see the comprehensive oxygen model of Stefan and Fang (1994) for a good example of this). In contrast, although inductive models often include some qualitative knowledge of the variables involved in relevant processes, they very rarely include any quantitative knowledge of the processes themselves, the functional forms of the model relationships being more usually constrained by the imagination of the modeller than by any of the preconceptions inherent in established scientific theories or laws.

The initial investigation of any natural phenomenon involves the employment of inductive reasoning. Without Kepler's painstaking inductive work in setting down his descriptive, empirical laws of planetary motion, for instance, it is unlikely that Newton would have been able to formulate his general laws of motion. Now that we have Newton's laws, however, no one would seriously suggest ignoring them and continuing to study planetary motion as Kepler did. Kepler's work resulted in a great leap forward for science; however, a second Kepler, ignoring Newton's work, would be unlikely to arouse much interest in the scientific community, and a second set of empirical laws of planetary motion, unconstrained by Newton's laws, would be unlikely either to be considered a substantial contribution to scientific knowledge or to be adopted in management strategy (for instance, for space shuttle operations). In the field of limnology it is no different. An initial inductive approach to each new general problem is justified and necessary. However, the continued formulation of different empirical models based on various new ensembles of lakes, and their subsequent publication in scientific journals (instead of as technical reports aimed at local environmental managers), may be less justifiable and less necessary. Inductive modelling, although a reasonable approach for obtaining estimates on a local level, is unlikely to result in progress in obtaining general solutions unless at some point a set of general principles, consistent with knowledge obtained in other contexts, can be generated.

In the particular case of hypolimnetic oxygen depletion (HOD), the time may have come to consider attempting to switch from an inductive to a deductive approach. It is now over 15 years since Mathias and Barica (1980), Charlton (1980), and Bossard and Gächter (1981), following Alsterberg's (1927) initial insight into the problem, suggested that oxygen depletion rates might profitably be resolved into a constant volume sink and an area sink proportional to the ratio of sediment area to water volume. This realization, which has had little impact on the structure of most inductive HOD models, would almost certainly be a core element of any deductive HOD model. However, Molot is of course correct in his opinion that the simple model presented by Livingstone and Imboden (1996) could be improved and extended by linking the area sink to some external driving variable such as the supply

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of a limiting nutrient. As stated in the original paper, the area sink reflects the sedimentation of organic matter smoothed over several years (Granéli 1978; Mathias and Barica 1980) and therefore is presumably related to long-term phosphorus (P) loading in P-limited lakes. Earlier lake eutrophication models (Imboden 1973, 1974) showed that HOD can be linked deductively to external P loading for a broad class of lakes with different depths and flushing times. However, even if an explicit link between the areal oxygen sink and an external forcing variable such as P loading were lacking, this would still not negate the potential that deductive models possess to evolve and progress by incorporating new knowledge of relevant processes as it becomes available. In contrast, inductive models neither have the capacity to advance the understanding of processes (and thus to improve predictive power) nor are they built to be extrapolated to cases which differ from the ensemble of cases that provided the data used to construct the model. Such a limitation has negative consequences for the guaranteed predictive accuracy Molot proposes as a criterion for a useful management tool; there is no guarantee whatsoever that an inductive oxygen depletion model will give an accurate prediction of oxygen profiles in lakes that differ from those on which the model is based. Molot himself, in fact, has acknowledged this limitation: Molot et al. (1992) admitted with respect to their inductive oxygen model that "it is not clear to what extent the model can be extrapolated beyond the range of conditions employed in this study."

Finally, then, as the opening sentence of Livingstone and Imboden (1996) makes clear, we agree completely with Molot when he says that we should advocate the use of both inductive and deductive approaches in the environmental sciences. We would add, however, that induction and deduction each has its place in the scheme of things; they are not interchangeable, and "personal predilection" is probably not a valid criterion for the use of either.

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Comment: Efficiency and bias of estimators and sampling designs for determining length–weight relationships of fishes[1]

You-Gan Wang

Introduction

Hayes et al. (1995) considered the efficiency and bias of the estimators in analysis of length–weight data. However, their suggestions and conclusions are somewhat misleading. I would like to clarify a few points on bias reduction.

The allometric model they used (their eq. 1) is

(1) $W = AL^B\xi$,

in which the error term and $\ln(\xi) \sim N(0, \sigma^2)$. This leads to a linear regression model after the logarithmic transformation,

(2) $Y = \ln(A) + BX + \varepsilon$,

where $X = \ln(L)$ and $Y = \ln(W)$. Note that under the model in eq. 1, $E(W) = AL^B \exp(\sigma^2/2)$, which differs from AL^B.

Parameter estimates

To minimize confusion, I will also use (B_0, B_1) to denote the estimates of $(\ln(A), B)$ (the intercept and the slope) obtained from ordinary linear regression. An estimate of A can be obtained from the inverse transformation $\hat{a}_{T1} = \exp(B_0)$. Hayes et al. (1995) incorrectly claimed that $\hat{a}_{T1}$ is an underbiased estimate of A (p. 86) and misleadingly suggested three methods ($\hat{a}_{T2}$, $\hat{a}_{T3}$, and $\hat{a}_{T4}$) to correct for the possible biases. The correction factors were originally developed by Miller (1984) and Finney (1951) for correcting for the bias in the mean of the untransformed dependent variable. Clearly, $\hat{a}_{T1}$ follows a log-normal distribution and

(3) $E(\hat{a}_{T1}) = A \exp(\sigma_0^2/2)$,

in which σ_0^2 is the variance of the estimate B_0. This implies that $\hat{a}_{T1}$ always overestimates A with a bias of $O(1/n)$ only. The correction factor $\exp(-\hat{\sigma}_0^2/2)$ may be applied to $\hat{a}_{T1}$ to correct for the bias, in which

(4) $$\hat{\sigma}_0^2 = \frac{\hat{\sigma}^2 \Sigma x_i^2}{n\Sigma x_i^2 - (\Sigma x_i)^2}$$

and $\hat{\sigma}^2$ is the residual variance.

Their Table 2 is even more confusing. The model is $E(W) = 6.59L^{3.12}/10^6$. Using eq. 1, their simulation model can be written as

(5) $W = 6.54l^{3.12} \exp(\varepsilon)/10^6$,

in which $\varepsilon \sim N(0, 0.0149)$ because $6.54 = 6.59 \exp(-0.0149/2)$. This implies that $A = 6.54/10^6$, which contradicts their statement that $A = 6.59/10^6$. In their simulation, Hayes et al. (1995) actually used the model

(6) $W = aL^B\eta$,

in which $\ln(\eta) \sim N(-\sigma^2/2, \sigma^2)$ so that $E(\eta) = 1$. This is a reparameterized version of the model in eq. 1 with $a = A \exp(\sigma^2/2) = 6.59/10^6$.

Both A and a may be of interest depending on the objective of the study and the interpretation of the error term (see the next section). Hayes et al. (1995) did not distinguish A and a explicitly and referred to them both as the intercept.

Obviously, $\exp(B_0)$ and $\exp(B_0 + \hat{\sigma}^2/2)$ are consistent estimates of A and a, respectively. A first-order bias-correction factor for both of these estimates is $\exp(-\sigma_0^2/2)$. Note that $\sigma_0 2$ is the variance of B_0 (of order $O(1/n)$) and $\hat{\sigma}^2$ is the residual variance, which in general does not tend to 0 as $n \to \infty$. As my simulation studies show, the first-order bias correction is sufficient even for small sample sizes (Table 1).

Hayes et al. (1995) also concluded that the nonlinear regression had lower precision than the log-transformation method. This is probably because the heterogeneous variance in nonlinear regression is not taken into account. If the data were generated from their eq. 3 with an additive error term

(7) $W = 6.59L^{3.12}/10^6 + \varepsilon$,

where $\varepsilon \sim N(0, 0.0149)$, I expect the reverse of their conclusion. Both the multiplicative model and the additive model are useful in practice. Therefore, one cannot conclude which one is better in general. The problem of interest is determining which error structure is more appropriate for the given data (see last paragraph).

Mean weight at length

If ε in eq. 2 represents measurement error, the true weight of the population at length L is AL^B. However, if ε represents the deviation of an individual growth curve from the population curve, the true weight of the population at length L is aL^B on the basis of eq. 6. In the latter case, model 1 is equivalent to assuming that each fish follows its own allometric function A_iL^B, in which A_i is a random effect following a lognormal

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Table 1. Mean estimates of A and a for haddock (using the simulation model of Hayes et al. 1995), $A = \exp(B_0)$ and $a = \exp(B_0 + \sigma^2/2)$, and their corresponding means after the first-order bias correction.

	Estimates of A ($\times 10^6$)		Estimates of a ($\times 10^6$)	
n	A	Corrected	a	Corrected
36	7.13	6.54	7.19	6.59
72	6.84	6.55	6.89	6.60
108	6.73	6.55	6.78	6.60
144	6.68	6.54	6.73	6.59
180	6.63	6.52	6.68	6.57
216	6.64	6.55	6.69	6.60
252	6.61	6.53	6.66	6.58
504	6.58	6.55	6.63	6.59
1008	6.55	6.53	6.60	6.58
2016	6.55	6.54	6.60	6.59

Note: The true value of A is $6.54/10^6$ and the true value of a is $6.59/10^6$. Results are based on 10 000 simulations with n fish in each simulation.

distribution and there is no measurement error in length or weight.

Let us come back to the model considered by Hayes et al. (1995). The parameter estimates of (B_0, B_1) can be used to estimate the mean weight at length (and other values as well). The model in eq. 1 suggests that the mean weight at length L is $W_L = AL^B \exp(\sigma^2/2)$. The estimated mean weight at length L using (B_0, B_1) is $W_0 = \exp(B_0)\, L^{B_1}$, which clearly follows a lognormal distribution, and $E(W_0) = AL^B \exp(\sigma_w^2/2)$, in which σ_w^2 is the variance of $\ln(W_0)$ given by

$$(8) \qquad \sigma_w^2 = \left(\frac{(x - \bar{x})^2}{\Sigma (x_i - \bar{x})^2} + \frac{1}{n} \right),$$

and $x = \ln(L)$. Therefore, a first-order bias-corrected estimate of W_L is

$$(9) \qquad W_1 = W_0 \exp((\sigma^2 - \sigma_w^2)/2) .$$

Note that the bias correction factor here is length specific and cannot be obtained by adjusting the intercept.

It seems that Hayes et al. (1995) intended to estimate a rather than A by adjusting an estimate of A. However, this is not desirable because (*i*) the adjusted A becomes an estimate of a that has a different meaning to A; (*ii*) even the bias-corrected estimate of a does not necessarily result in a reasonable estimate of the mean weight at length, as reported by Hayes et al. (1995) (pp. 88 and 89); (*iii*) a simple and more accurate analytic expression is available (eq. 9) to correct for the possible bias in the predicted mean weight at length (and the correction factor is length specific and accounts for the correlation between the two parameter estimates B_0 and B_1); and (*iv*) adjusted parameter estimates may be suitable for one purpose but not for the others (the allometric parameters are not used only for estimating the mean weight at length), and therefore correction factors should be derived from the statistics or parameters of interest.

The model given by eq. 1 takes account of the possible deviation of individual fish from the population but does not incorporate measurement error or possible model error. A more realistic model is to assume both individual variability and measurement error. An appropriate weighting function will be required to obtain the most efficient analysis. By plotting the residuals against the predicted values or x values, one may find an appropriate weighting function to obtain more efficient estimates. Correction factors in this case will require one to partition the residuals into model error owing to individual variability and to measurement error. To achieve this, longitudinal data (multiple length–weight data from individuals) are necessary, otherwise the error term ε_i will be confounded with individual variability. Another important factor is the measurement error in length, which is often ignored in practical analysis. The amount of this measurement error varies depending on how the data are collected. However, my simulation results indicate that substantial biases may occur even when this error is about 1%. Although it is not easy (if not impossible) in many cases to take account of measurement errors in the analysis, their great impact should be borne in mind in real data analysis.

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Reply: Efficiency and bias of estimators and sampling designs for determining length–weight relationships of fish[1]

Daniel B. Hayes and Jon K.T. Brodziak

In his comment on methods for reducing bias in the estimation of parameters for the allometric equation describing length–weight data, Wang (1997) makes several extensions to methods available in the theoretical literature. In particular, his point that the bias in predicted mean weight depends (in part) on the standard error of the prediction (and hence on length) is potentially important. In our simulations, the variance of predicted mean weight was quite low and produced negligible bias; in other applications of log-transformed data with higher variance (e.g., catch-curve analyses), this component would have a noticeable effect. Wang (1997) also correctly points out that measurement error in lengths (which in this application is treated as being zero) could introduce bias into estimates of both the slope and intercept. Wang does not, however, provide an indication of the amount of measurement error that generally occurs in length measurements. Thus, it is difficult to evaluate the magnitude of bias that would occur in general practice.

The primary area that Wang and we appear to disagree is the interpretation of the two allometric relationships he denotes as

(1) $W = AL^B\xi$

where $\ln(\xi) \sim N(0, \sigma^2)$ and

(2) $W = aL^B\eta$

where $\ln(\eta) \sim N(-\sigma^2/2, \sigma^2)$. Wang (1997) correctly asserts that eq. 2 is a reparameterized version of eq. 1 with $a = A\exp(\sigma^2/2)$. We feel that the essential difference is that in eq. 2 the expected value of η is equal to 1, whereas in eq. 1 the expected value of ξ depends on the underlying variance. One of the key points highlighted in Hayes et al. (1995) is that the model $W = AL^B\xi$ does not represent the mean response of weight as a function of length, but rather passes through the geometric mean (Fig. 1), which for the lognormal distribution is equivalent to the median. The shift in the object of the regression from the mean to the geometric mean has long been known (e.g., Finney 1941) and is the rationale given by several authors (e.g., Goldberger 1968; Miller 1984; Gutreuter 1990) for the correction factor of $\exp(\sigma^2/2)$ to adjust for transformation-induced bias in the linearized form of the allometric equation.

We agree with Wang's suggestion that $\exp(-\sigma_0^2/2)$ is an appropriate correction factor for A. In fact, Goldberger (1968) suggested the same correction factor but importantly emphasized that estimates of A are actually based on the median response rather than the mean response of the dependent variable. Thus, the essence of the problem for applied statisticians is to decide which characteristic of the independent variable they seek. We feel that, in general, analysts intend to determine mean response or mean level of the dependent variable (in this case weight). Wang (1997) asserts that $\exp(-\sigma_0^2/2)$ is an appropriate first-order bias-correction factor for a as well as A. While this may be an appropriate first-order correction factor, the evidence provided by Hayes et al. (1995) suggests that first-order approximations are insufficient to adequately adjust for the effects of transformation bias.

Wang (1997) is correct that the function we used to generate the data for the simulations followed his eq. 2. The reason for this should be obvious: to generate weight at length data with the specified mean and variance, one cannot use eq. 1 with A set to the parameter representing the deterministic allometric equation. In other words, if A were set to 6.59×10^{-6} (which was the intercept for the actual curve connecting the arithmetic means) as we used in our simulation and generated random deviates by multiplying by the factor $\exp(N(0, 0.0149))$, this would result in weight at length observations with a mean that is greater than the desired value. Thus, one of the critical steps in our simulations was to adjust the mean and variance of the random normal deviates used to introduce variability into the generated data to insure that the actual means of the weight at length data fell on the deterministic line $W = 6.59 \times 10^{-6} L^{3.120}$. The apparent paradox in this situation is that stochasticity cannot be added to the deterministic line connecting the actual means simply by multiplying the deterministic line by a random variate generated by $\exp(N(0, \sigma^2))$. Unfortunately, when we apply the log transformation in practice, we unwittingly create a statistical model that assumes this to be true. Thus, the normal practice results in an inconsistent estimator (i.e., biased) with respect to the actual curve relating mean weight to mean length. This realization was partly the reason we explored the application of nonlinear regression for estimating the parameters of the allometric equation. Our hope was that some of the problems with log transformations could be avoided by using a nonlinear regression. Unfortunately, the nonlinear models

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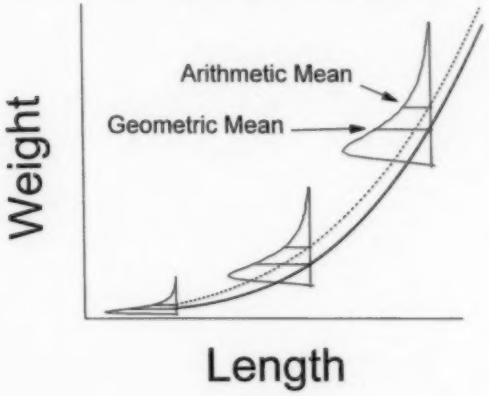
Fig. 1. Curves representing the objects of the retransformed regression equations $W = AL^B\xi$ passing through the geometric mean (solid line), and $W = aL^B\eta$ passing through the arithmetic mean (broken line). The difference between the geometric and arithmetic mean lines has been enlarged in the illustration for clarity.



we applied were less precise than the log-transformation method. While Wang (1997) is correct that the lower precision of the nonlinear regression was probably due to the heterogeneous variance we generated in weight at length, heteroskedasticity is an obvious feature of any real-life length–weight data sets that span a sufficiently broad length interval. As such, it would not be realistic to generate weight at length data with a constant additive error term. In fact, preliminary simulations where we attempted to do this resulted in the generation of fish with negative weights, clearly an impossibility.

In summary, our motivation for exploring the various correction methods proposed in the theoretical literature was that, although many of the methods proposed are based on sound analytic methods, nearly all depend to some extent on approximations or on asymptotic properties of estimators. The difficulty for applied statisticians is in choosing among the various alternatives proposed. We feel that the advice we provided is reasonable for practicing fishery biologists but also hope that this provides a basis for further exploration into estimation problems posed by transformation methods in general.

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